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Synthesis of cyclophospho-glucoses and glucitols

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Abstract—The syntheses of cyclophosphodiesters of 5-C-(hydroxymethyl)-hexoses and hexitols and of 6-C-(hydroxymethyl)-hexoses are reported, along with that of 6-deoxy-*gluco*-heptose 7-phosphate. These compounds proved to be reasonable substrate mimics and show inhibitory activity against human D-*myo*-inositol 3-phosphate synthase.

1. Introduction

In some prokaryotic and most eukaryotic organisms, major cellular events are controlled by inositol-containing compounds; these include events such as signal transduction and secondary messenger signalling,[1,2] stress response and cell wall biogenesis.[3–5] The de novo synthesis of all inositol-containing compounds involves a rate-limiting step, which is the conversion of D-glucose 6-phosphate (**1**, G6P) into D-*myo*-inositol 3-phosphate (also named L-*myo*-inositol 1-phosphate) catalysed by MIP synthase (D-*myo*-inositol 3-phosphate synthase; EC 5.5.1.4). For this isomerisation step, MIP synthase uses β-nicotinamide adenine dinucleotide (NAD) as a prosthetic group.[6]

The commonly accepted mechanism (Scheme 1) involves the reaction intermediate 5-keto-glucose 6-phosphate **4** generated by oxidation of the C-5-hydroxyl of the open-form of G6P **1**, i.e., **3**, with the concomitant reduction of NAD to NADH, followed by the enolisation and aldol condensation of **5** to yield the inosose phosphate **6**. This reaction intermediate is subsequently reduced by NADH to produce MIP and regenerate NAD.[7] Several mechanistic studies indicate that MIP synthase binds the open form of G6P **1** and that this binding nucleates the active enzyme folding.[8]

The human MIP synthase (G6P **1**, K_m=0.57 mM) shares high sequence and structural homology with the yeast enzyme (G6P **1**, K_m=1.18 mM) for which Geiger reported the crystal structures of MIP synthase bound to two different inhibitors (2-deoxy D-glucitol 6-phosphate **7** (yeast K_i=2.3 μM) and 6-deoxy-D-glucitol 6-(*E*)-vinyl phosphonate **8** (yeast K_i=0.67 μM), Scheme 2).[9,10] Amongst other important residues, a series of four lysine side chains were identified as playing a major role in complex-stabilisation through hydrogen-bond interactions with the sugar hydroxyls, and ionic interactions with the phosphate moiety of G6P (**1**). These analyses also established that the yeast enzyme was capable of binding the phosphorylated and phosphonylated sugar derivatives **7** and **8**, in the same binding pocket but in almost opposite orientations.[11,12] This behaviour is thought to be facilitated by the flexibility and charge stabilisation brought on by the lysine side chains. Furthermore, the phosphonate **8** is the most potent inhibitor of MIP synthase while its *Z*-isomer showed no inhibitory properties. This potency is achieved through a slowly-reversible competitive binding and oxidation catalysed by the NAD-bound MIP synthase. These observations led to the proposal that the enolisation step could be intramolecularly catalysed by the phosphate moiety itself (Scheme 2).[10]

A major structural difference between the phosphonate **8** and the glucitol phosphate **7** is the introduction of rigidity in the phosphonate region due to the conjugated alkene system. Therefore, we wished to explore whether improved potency over that of phosphate **7** could be achieved by introducing rigidity in the phosphate region and whether 'productive' binding could result. To this end, five cyclophosphate-containing hexoses and hexitols (**9–13**; Scheme 3) have been synthesised, as inhibition studies on MIP synthase have also shown that the 2-deoxy-sugar and the glucitol series of substrate analogues display dramatically enhanced inhibition properties when compared to that of their sugar parents. Unlike the cyclophosphodiesters of 5-C-(hydroxymethyl)-hexoses (**9–12**), the five-membered ring cyclic

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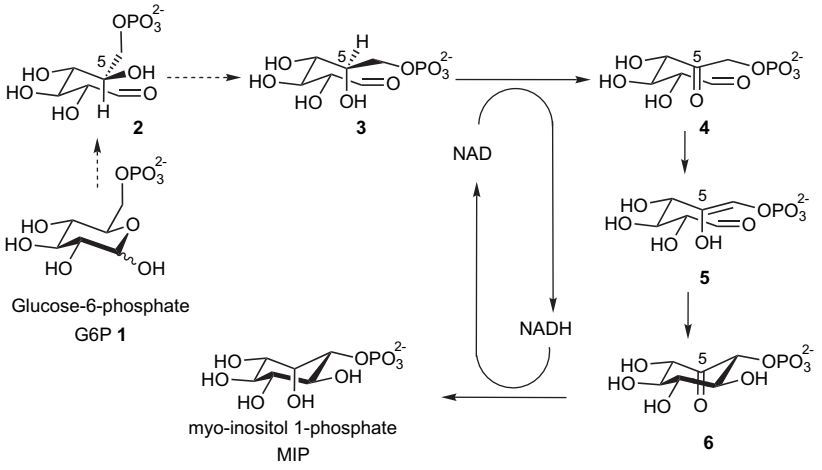
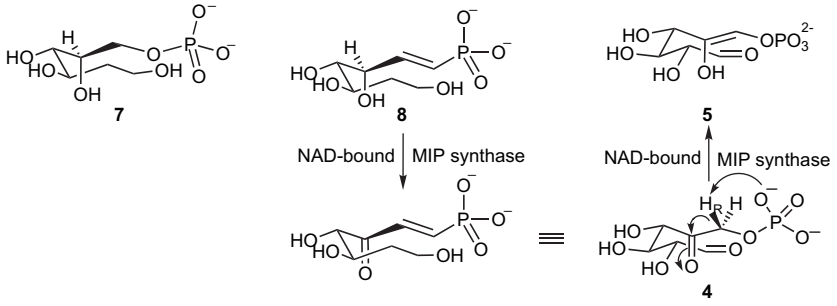
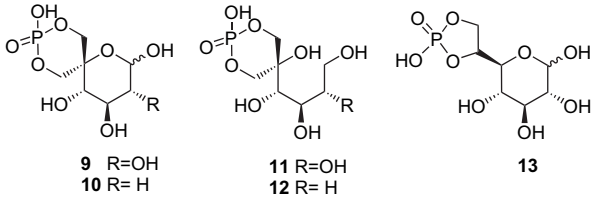


Scheme 1. MIP synthase mechanism.



Scheme 2. Known potent MIP synthase inhibitors and phosphate-catalysed enolisation of 5-ketoglucose 6-phosphate.

phosphate **13**, when in the open form, possesses a secondary C-5 hydroxyl group. This hydroxyl could potentially be oxidised to a keto-sugar once bound to the NAD–MIP synthase complex, hence this becomes a mimic of the hydrogen-abstraction transition state occurring during the enolisation step, if such step is indeed phosphate-catalysed.



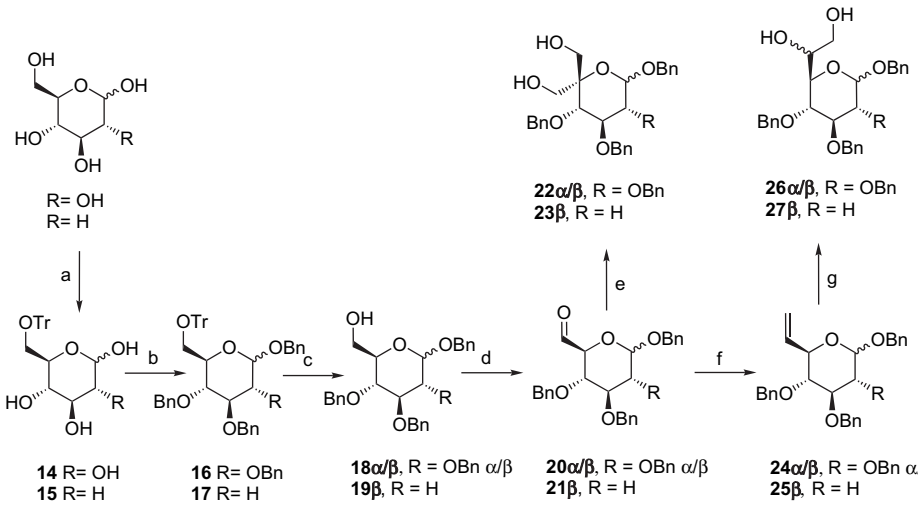
Scheme 3. Cyclophospho-glucoses and glucitols.

2. Results and discussion

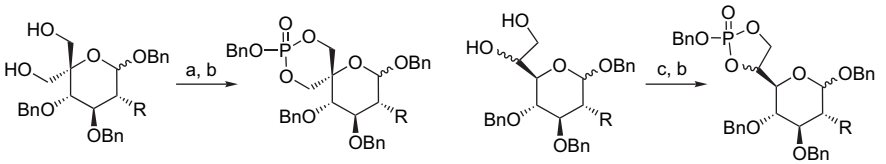
The aldehydes **20** and **21**, precursors to the 5-C-(hydroxymethyl)-hexoses **9**–**12**, were obtained from D-glucose and 2-deoxy-D-glucose in 33% and 17% overall yield, respectively. Glucose and 2-deoxy-glucose were selectively tritylated to yield the partially protected sugars **14** and **15**, which were subsequently benzylated (**16** and **17**) and selectively detritylated to yield the primary alcohols **18** and **19**, which were readily converted under Swern conditions to the aldehydic synthetic intermediates **20** and **21** (Scheme 4). The 1,3 diols, **22** and **23**, were accessed via an aldol-type condensation with formaldehyde of the aldehydic sugar precursors, **20** and **21**, respectively), followed by a spontaneous Canizzaro reduction, as first developed by Schaeffer[13] and applied to the synthesis of 5-C-(hydroxyoxymethyl)-D-xylohexopyranoside by Hiler.[14]

Direct Wittig olefination of the aldehydic glucosides **20** and **21** afforded the methylene intermediates **24** and **25**, which were subsequently dihydroxylated to yield the 6-C-(hydroxymethyl)-hexoses **26** and **27**. In order to establish the chirality at the C-6 carbon of **26** and **27**, derivatisation of **26β** with an acetonide group was carried out and offered a mixture of diastereoisomers in a 15:85 ratio (established by ^{1}H NMR). Selective crystallisation in dichloromethane/hexane afforded crystals of the major isomer and crystallographic analyses confirmed the C-6 carbon configuration as *R*.

We previously reported the use of benzyloxy-bis(diisopropylamino)phosphine as phosphitylating agent of 1,2- and 1,3-diols.[15] However, the use of the more reactive and rarely used benzyloxydichlorophosphine in the presence of an excess of triethylamine was necessary to achieve quantitative phosphitylation of the 1,2- and 1,3-diol sugars, **26/27** and **22/23**, respectively (Scheme 5). Each cyclic phosphite triester intermediate, **28α**, **28β** and **29** was isolated as a mixture of diastereoisomers ((*R*)-P and (*S*)-P) in a 1:1 ratio. All three cyclic phosphite triesters proved to be too unstable to allow for extensive chromatographic purification and separation of



Scheme 4. Divergent syntheses of the 1,2- and 1,3-diols. (a) TrCl, pyridine, 70 °C, **14**: 97%, **15**: 58%; (b) BnBr, NaH, Bu_4NI, DMF, **16**: 82%, **17**: 80%; (c) $AlCl_3$, DCM, Et_2O, **18**: 58%, **19**: 73%; (d) DMSO, $(COCl)_2$, Et_3N, DCM, −78 °C, quant.; (e) 1,4 dioxane, formaldehyde, NaOH, rt, **22α/β**: 69%, **23β**: 50%; (f) Ph_3PCH_3Br, BuLi, THF, −78 °C to rt, **24α/β**: 44%, **25β**: 50% and (g) OsO_4, NMO, DCM, **26α/β**: 100%, **27β**: 98%.



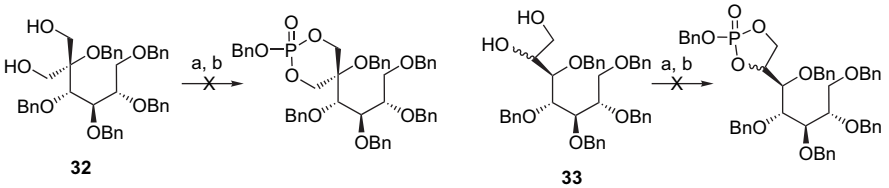
Scheme 5. Synthesis of the phosphate triesters. (a) $(BnO)PCl_2$, Et_3N, DCM; (b) *t*-BuOOH, DCM and (c) 2,4-DNP, $(BnO)P(N^iPr)_2$, DCM.

the individual diastereoisomers. Subsequent oxidation of the diastereoisomeric mixture using anhydrous *tert*-butyl hydroperoxide afforded the corresponding cyclic phosphate triester diastereoisomers in high yields. The diastereoisomeric mixtures, while cumbersome for characterisation purposes, could be used as such for the subsequent steps since both the phosphorus chirality and the anomeric stereochemistry were to be lost at the deprotection step.

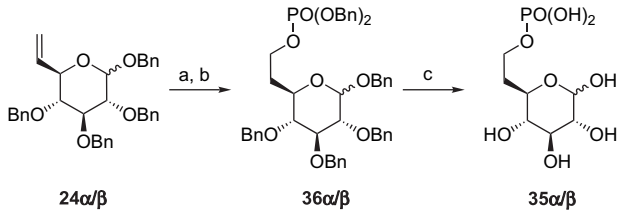
It must be noted that attempts to prepare the cyclic phosphates from the glucitol parents of **22** and **26**, i.e. glucitols **32** and **33** (Scheme 6) only yielded to the detection of phosphate monoesters, with less than 10% cyclised phosphite triesters identified by ^{31}P NMR.

Additionally, the 6-deoxy-*gluco*-heptose-7-phosphate **35** was synthesised from intermediate **24** (Scheme 7) using 9-BBN for the regioselective hydroxylation of the alkene bond and tetrabenzylpyrophosphate in the presence of NaH for the phosphorylation of the resulting primary alcohol to yield the fully benzylated 6-deoxy-*gluco*-heptose 7-phosphate **36**.

The debenzylation of **28**, **29**, **30**, **31** and **36** proved to be less straightforward than anticipated. Pd-catalysed hydrogenation in methanol in the presence of a molar equivalent of inorganic base was required for the debenzylation of cyclic phosphotriesters and quantitative conversion to the cyclic phosphate diester sugars with no cyclophosphate ring opening.[15] The protected sugars **28**, **29** and **36** required two reaction cycles with filtration of the catalyst and new addition of Pd/C and 0.5 equiv K_2CO_3 over the course of the reaction, to yield quantitatively the cyclic phosphate diesters **9**, **10** and **35**, respectively. Deprotection of the cyclic phosphate triester **30** to cyclic phosphate diester **13** was only achieved when Birch reduction conditions were employed. Unfortunately, when applied to **31**, neither of these sets of conditions afforded the fully deprotected cyclic phosphate diester with



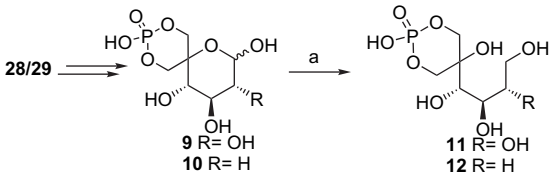
Scheme 6. Attempted syntheses of the cyclic phosphate triesters from the glucitols **32** and **33**. (a) $(BnO)PCl_2$, Et_3N, DCM and (b) *t*-BuOOH, DCM.



Scheme 7. Synthesis of 6-deoxy-*gluco*-heptose-7-phosphate. (a) i. 9-BBN, THF; ii. H_2O_2, NaOH, 97%; (b) NaH, TBPP, 42% and (c) H_2, Pd/C, MeOH, quant.

sufficient level of purity to allow for isolation and full characterisation.

Since it was not possible to access the cyclic phosphate triesters glucitol-derivatives from the glucitol diol precursors **32** and **33** (Scheme 6), the glucose and 2-deoxy glucose cyclic phosphates **9** and **10** were treated with an excess of sodium borohydride in water to access the glucitols **11** and **12**, respectively (Scheme 8). Surprisingly, no ring opening of the cyclic phosphate diesters was detected, while the hemiacetal carbonyl was reduced quantitatively.



Scheme 8. Synthesis of the glucitol cyclic phosphates **11** and **12** from the glucose parents. (a) $NaBH_4$, H_2O then Dowex H^+.

The spirocyclophosphates **9**–**12** and 6-deoxy-*gluco*-heptose 7-phosphate **35** were then tested for activity against MIP synthase. When compounds **9**–**12** were tested against purified human MIP synthase,[16] only inhibition was observed at low millimolar concentration (Table 1), the 2-deoxy cyclic phosphates **10** and **12** possess better affinity for the enzyme than their glucose parent. Unlike 2-deoxy-glucitol 6-phosphate **7**, known to inhibit eukaryotic MIP synthase at low micromolar concentrations,[9,10] the spirophosphate derived from 2-deoxyglucitol **12**, had limited affinity for the human enzyme with a 10-fold loss in potency. This could indicate that the charge and the conformational flexibility of the phosphate moiety are crucial for recognition, binding and inhibition of the human enzyme. Surprinsingly, no inhibition was detected for the homoglucose 7-phosphate **35**. It is to be noted that the increased chain length of the sugar moiety resulted in complete loss of inhibition while shorter sugar phosphates (tetrose and pentose derivatives) are known MIP synthase inhibitors. Finally, the kinetic studies of the effect of the five-membered ring cyclic phosphate **13** have so far been inconclusive. Further enzyme kinetic experiments and compound stability studies are needed to establish the inhibitory potency of this glucose 6-phosphate mimic.

Table 1. Inhibition constant values of spirocyclophosphates **9**–**12** and 6-deoxy-*gluco*-heptose 7-phosphate **35** against human MIP synthase determined at a G6P concentration of 5 mM

	IC_{50} (human MIP synthase; K_{mG6P}=0.57 mM)	Calculated K_i; K_i=IC_{50}/(1+[G6P]/K_m)
9	No inhibition at 5 mM	N/A
10	0.6 mM	32 μM
11	0.85 mM	46 μM
12	0.56 mM	30 μM
35	No inhibition at 5 mM	N/A

3. Conclusions

In conclusion, we have synthesised five novel C7-sugar cyclophosphates, analogues of glucose 6-phosphate in addition to 6-deoxy-*gluco*-heptose 7-phosphate and evaluated these compounds against human MIP synthase. While conformational strain was introduced into the molecular scaffold in a hope to increase conformational likeness to a reaction intermediate, no improvement in MIP synthase inhibition was observed when compared to the known inhibitors.

4. Experimental

4.1. General experimental details

HPLC runs were performed on a Gilson Anachem system with UV detector operating at 254 nm using a C18-RP Supelcosyl HPLC column. IR spectra were recorded on a Perkin Elmer spectrum RI I FT-IR system; absorption in cm^{-1}. Mass spectra were obtained on an LCT premier Micromass® Technologies Waters (ASEP Belfast). Optical rotations were determined with a Perkin Elmer 341 polarimeter. The ^1H, ^{13}C and ^{31}P NMR spectra were recorded in $CDCl_3$, D_2O, CD_3OD or $(CD_3)_2SO$ on a Bruker AC 300 MHz spectrometer. HMQC and ^1H–^1H COSY experiments were performed on a Bruker AMX 500 spectrometer. TMS (0 ppm, ^1H NMR), $CDCl_3$ (77 ppm, ^{13}C NMR) and triethyl phosphate (0.2 ppm, ^{31}P NMR) were used as internal references. The chemical shifts (δ) are reported in parts per million and the coupling constants (J values) are recorded in hertz. The hydrogen and carbon assignments were done when 2D experiments (^1H–^1H COSY and ^1H–^{13}C HMQC experiments) allowed it. The benzylic methylene hydrogens are described as two doublets instead of AB systems for clarity purposes.

4.1.1. 6-*O*-Trityl-D-glucopyranose 14.[17] To a solution of D-glucose (10 g, 55.5 mmol) in dry pyridine (120 mL) was added triphenylmethyl chloride (17 g, 61.0 mol). The resulting suspension was then heated at 75 °C for 18 h. The reaction mixture was then allowed to cool down to room temperature. Pyridine was removed under reduced pressure to afford a yellow gum. This residue was dissolved in DCM (150 mL) and washed with NH_4Cl (100 mL), $NaHCO_3$ (100 mL) and brine (100 mL). The organic layer was the dried, filtered and concentrated under reduced pressure to afford the crude 6-*O*-trityl-D-glucose as a yellow oil (40 g). Purification by flash chromatography (100/0→90/10, v/v, $CHCl_3$/EtOH) afforded the pure compound **15** (mixture of anomers) as a white foam (22.0 g, 94%). ν_{max} (liquid film) 3392, 3018, 1597, 1490, 1047 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.45–7.10 (15H, m, Ar), 5.69–4.50 (4H, m), 3.88 (2H, m), 3.47–3.22 (5H, m); ^{13}C NMR ($CDCl_3$) δ 143.7, 143.6, 128.7,

127.8, 127.1, 127.0, 96.3, 92.3, 87.0, 86.7, 74.5, 73.4, 72.0, 71.3, 70.3, 64.0, 63.5; HRMS (ES) Calcd for $C_{25}H_{25}O_6$ ($M-H^+$) 421.1651 found 421.1654.

4.1.2. Benzyl 6-*O*-trityl-2,3,4-tri-*O*-benzyl-D-glucopyranoside 16.[18] Sodium hydride (1.42 g of 60% suspension in mineral oil, 35.55 mmol) was added portionwise to a solution of 6-*O*-trityl-D-glucopyranoside **14** (3.0 g, 7.11 mmol), benzyl bromide (4.2 mL, 35.55 mmol) and tetrabutylammonium iodide (264 mg, 0.71 mmol) in dry DMF (80 mL) at 0 °C. The resulting suspension was allowed to warm up to room temperature and stirred overnight. The reaction mixture was then diluted with Et_2O (100 mL) and $NH_4Cl_{(aq)}$ (100 mL) was added to the resulting solution. The organic layer was washed with $NH_4Cl_{(aq)}$ (50 mL), water (50 mL), brine (50 mL), dried, filtered and concentrated under vacuum to afford the crude sugar as a waxy yellowish solid (6.61 g). Purification by flash chromatography (100/0→50/50, v/v, PE/EtOAc) afforded the pure compound **16** as a waxy solid (4.52 g, 82%). ν_{max} (liquid film) 3028, 2926, 1955, 1659, 1450, 1077, 756 cm^{-1}; $[\alpha]_D^{20}$ +17.3 (*c* 0.03, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.54–7.16 (33H, m, Ar), 6.86 (2H, appd, *J* 6.6 Hz), 5.07 (0.6H, d, *J* 11.8 Hz, β, CH_2–Ph), 5.01 (0.6H, d, *J* 10.9 Hz, β, CH_2–Ph), 4.97 (0.4H, d, *J* 10.7 Hz, α, CH_2–Ph), 4.95 (0.4H, d, J_{1-2} 3.7 Hz, α, H-1), 4.89 (0.6H, d, *J* 10.8 Hz, β, CH_2–Ph), 4.82–4.68 (3.8H, m, β/α, CH_2–Ph), 4.63 (0.4H, d, *J* 12.2 Hz, α, CH_2–Ph), 4.61 (0.6H, d, *J* 12.0 Hz, β, CH_2–Ph), 4.55 (0.6H, d, J_{1-2} 7.2 Hz, H-1), 4.36 (0.6H, d, *J* 10.4 Hz, β, CH_2–Ph), 4.30 (0.4H, d, J_{4-5} 10.4 Hz, α, CH_2–Ph), 4.02 (0.4H, t, $J_{3-4,4-5}$ 9.3 Hz, α, H-4), 3.87 (0.4H, ddd, J_{5-6} 1.5 Hz, J_{4-5} 4.3 Hz, $J_{5-6'}$ 10.4 Hz, α, H-5), 3.81 (0.6H, t, $J_{3-4,4-5}$ 9.2 Hz, β, H-4), 3.68–3.58 (2.6H, m, β/α, H-2, β/α, H-3, β, H-6), 3.46 (0.4H, dd, $J_{6-6'}$ 1.8 Hz, J_{5-6} 10.1 Hz, α, H-6), 3.42 (0.6H, ddd, J_{5-6} 1.7 Hz, $J_{5-6'}$ 3.9 Hz, J_{4-5} 9.8 Hz, β, H-5), 3.27 (0.6H, dd, $J_{5-6'}$ 4.1 Hz, $J_{6-6'}$10.1 Hz, β, H-6′), 3.19 (0.4H, dd, $J_{5-6'}$ 4.5 Hz, $J_{6-6'}$ 10.1 Hz, α, H-6′); ^{13}C NMR ($CDCl_3$) δ 144.0, 138.8, 138.6, 138.3, 138.0, 137.9, 137.4, 137.1, 128.9, 128.7, 128.5, 128.4 (2), 128.2 (2), 128.1, 127.8, 127.7 127.6, 127.0, 102.3 (β, C-1), 94.7(α, C-1), 86.4 (β/α, *C*(Ph)$_3$), 84.8 (β, C-2 or C-3), 82.6 (β, C-3 or C-2), 82.4 (α, C-4), 80.3 (α, C-2 or C-3), 78.2 (α, C-2 or C-3), 78.0 (β, C-4), 76.0 (β/α, 2, O–*C*H_2–Ph), 75.0 (β/α, 2, O–*C*H_2–Ph), 74.7 (C-5), 73.0 (β/α, O–*C*H_2–Ph), 70.7 (β/α, O–*C*H_2–Ph), 70.6 (C-5), 68.7 (β/α, O–*C*H_2–Ph), 62.5 (C-6); LRMS (ES) ($M+Na^+$) 783.0.

4.1.3. Benzyl 2,3,4-tri-*O*-benzyl-D-glucopyranoside 18.[18] To a solution of benzyl-6-*O*-trityl-2,3,4-tri-*O*-benzyl-D-glucopyranoside **16** (4.27 g, 5.81 mmol) in dry DCM (60 mL) was added aluminium trichloride (0.78 g, 5.81 mmol) diluted in dry Et_2O (60 mL). The resulting solution turned bright yellow almost immediately. The reaction mixture was then stirred for 1 h. The reaction mixture was then diluted with Et_2O (100 mL) and $NaHCO_3$ (100 mL). The aqueous layer was then extracted with Et_2O (75 mL×2). The combined organic layers were then washed with $NaHCO_3$ (70 mL×2), water (70 mL×2) and brine (70 mL×2), dried, filtered and concentrated under reduced pressure to afford the crude sugar (4.32 g) as a colourless oil, which turned into a waxy solid overnight. Purification by flash chromatography (90/10→50/50, v/v, PE/EtOAc) afforded three fractions of pure sugar: fraction 1 (834 mg) α-anomer, fraction 2 (681 mg) mixture of anomers and fraction 3 (327 mg) β-anomer (overall yield 58%). β-anomer: ν_{max} (liquid film) 3469, 2925, 1497, 1360, 1214, 1070 cm^{-1}; $[\alpha]_D^{20}$ −8.4 (*c* 0.028, $CHCl_3$) (lit.[18b] $[\alpha]_D^{20}$ −10 (*c* 1, $CHCl_3$)); ^1H NMR ($CDCl_3$) δ 7.37–7.25 (20H, m, Ar), 4.95 (1H, d, *J* 10.5 Hz, CH_2–Ph), 4.93 (1H, d, *J* 11.9 Hz, CH_2–Ph), 4.92 (1H, d, *J* 11.0 Hz, CH_2–Ph), 4.86 (1H, d, *J* 11.0 Hz, CH_2–Ph), 4.80 (1H, d, *J* 10.9 Hz, CH_2–Ph), 4.73 (1H, d, *J* 10.9 Hz, CH_2–Ph), 4.69 (1H, d, *J* 11.8 Hz, CH_2–Ph), 4.63 (1H, d, *J* 11.0 Hz, CH_2–Ph), 4.57 (1H, d, J_{1-2} 7.8 Hz, H-1), 3.87 (1H, dd, J_{5-6} 2.7 Hz, $J_{6-6'}$ 5.7 Hz, H-6), 3.73–3.65 (1H, m, H-6′), 3.67 (1H, appt, $J_{3-4,4-5}$ 9.0 Hz, H-4), 3.57 (1H, appt, $J_{2-3,3-4}$ 9.3 Hz, H-3), 3.49 (1H, appt, $J_{1-2,2-3}$ 8.6 Hz, H-2), 3.36 (1H, ddd, J_{5-6} 2.8 Hz, $J_{6-6'}$ 4.6 Hz, J_{4-5} 9.6 Hz, H-5); ^{13}C NMR ($CDCl_3$) δ 138.5 (Ar), 138.3 (Ar), 138.0 (Ar), 137.3 (Ar), 128.5 (Ar), 128.3 (2, Ar), 128.1 (Ar), 128.0 (Ar), 127.9 (Ar), 127.8 (Ar), 127.7 (Ar), 127.6 (Ar), 102.8 (C-1), 84.5 (C-3), 82.3 (C-2), 77.6 (C-4), 75.7 (*C*H_2–Ph), 75.1 (2, *C*H_2–Ph), 75.0 (C-5), 71.7 (*C*H_2–Ph), 62.1 (C-6); HRMS (ES) Calcd for $C_{34}H_{35}O_6$ ($M-H^+$) 539.2434 found 539.2440; α-anomer: ν_{max} (liquid film) 3469, 2925, 1497, 1360, 1214, 1070 cm^{-1}; $[\alpha]_D^{20}$ +36.4 (*c* 0.018, $CHCl_3$) (lit.[18a] $[\alpha]_D^{20}$ 63.4 (*c* 1.5, $CHCl_3$)); ^1H NMR ($CDCl_3$) δ 7.40–7.24 (20H, m, Ar), 5.01 (1H, d, *J* 10.9 Hz, CH_2–Ph), 4.88 (1H, d, *J* 11.0 Hz, CH_2–Ph), 4.84 (1H, d, *J* 10.9 Hz, CH_2–Ph), 4.81 (1H, d, J_{1-2} 3.6 Hz, H-1), 4.68 (2H, 2d, *J* 11.7 Hz, 12.9 Hz, CH_2–Ph), 4.64 (1H, d, *J* 11.0 Hz, CH_2–Ph), 4.55 (1H, d, *J* 11.9 Hz, CH_2–Ph), 4.54 (1H, d, *J* 12.3 Hz, CH_2–Ph), 4.07 (1H, appt $J_{2-3,3-4}$ 9.3 Hz, H-3), 3.72–3.66 (3H, m, H-6, H-6′, H-5), 3.54 (1H, appt, $J_{3-4,4-5}$ 9.2 Hz, H-4), 3.50 (1H, dd, J_{1-2} 3.4 Hz, J_{2-3} 9.4 Hz, H-2); ^{13}C NMR ($CDCl_3$) δ 138.8 (Ar), 138.1 (Ar), 137.1 (Ar), 128.4 (Ar), 128.3 (Ar), 128.0 (Ar), 127.9 (Ar), 127.7 (Ar), 127.5 (Ar), 95.6 (C-1), 81.9 (C-3), 80.0 (C-2), 77.4 (C-4), 75.7 (*C*H_2–Ph), 75.0 (*C*H_2–Ph), 73.0 (*C*H_2–Ph), 71.0 (C-5), 69.2 (*C*H_2–Ph), 61.8 (C-6); HRMS (ES) Calcd for $C_{34}H_{35}O_6$ ($M-H^+$) 539.2434 found 539.2441.

4.1.4. 6-*O*-Trityl-2-deoxy-D-glucopyranose 15.[19] To a solution of 2-deoxy-D-glucose (1 g, 6.1 mmol) in dry pyridine (20 mL) was added triphenylmethyl chloride (1.86 g, 6.7 mol). The resulting suspension was then heated at 75 °C for 18 h. The reaction mixture was then allowed to cool down to room temperature. Pyridine was removed under reduced pressure to afford a yellow gum. This residue was dissolved in DCM (50 mL) and washed with NH_4Cl (30 mL), $NaHCO_3$ (30 mL) and brine (30 mL). The organic layer was the dried, filtered and concentrated under reduced pressure to afford the crude 6-*O*-trityl-2-deoxy-D-glucose as a yellow oil (4.2 g). Purification by flash chromatography (100/0→90/10, v/v, $CHCl_3$/EtOH) afforded the pure **15** (mixture of anomers) as a white foam (1.45 g, 58%). ν_{max} (liquid film) 3391, 3017, 1597, 1448, 1063 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.45–7.17 (15H, m, Ar), 5.26 (0.7H, br s), 4.71 (0.3H, d, *J* 9.2 Hz), 4.01–3.76 (2H, m), 3.41–3.33 (3H, m), 2.18–1.97 (1H, m), 1.63–1.43 (1H, m); ^{13}C NMR ($CDCl_3$) δ 143.6 (2), 143.4, 128.6, 128.5, 127.9, 127.8, 127.2, 127.1, 94.1, 91.9, 87.3, 87.0, 741, 73.8, 71.2, 70.2, 64.7, 64.4, 39.5, 37.0; HRMS (ES) Calcd for $C_{25}H_{25}O_5$ ($M-H^+$) 405.1702 found 405.1721.

4.1.5. Benzyl 6-*O*-trityl-3,4-di-*O*-benzyl-2-deoxy-D-glucopyranose 17. Sodium hydride (552 mg of 60% suspension

in mineral oil, 13.8 mmol) was added portionwise to a solution of 6-*O*-trityl-2-deoxy-D-glucopyranoside **15** (1.4 g, 3.45 mmol), benzyl bromide (1.64 mL, 13.8 mmol) and tetrabutylammonium iodide (130 mg, 0.35 mmol) in dry DMF at (40 mL) 0 °C. The resulting suspension was allowed to warm up to room temperature and stirred overnight. The reaction mixture was then diluted with Et_2O (75 mL) and $NH_4Cl_{(aq)}$ (75 mL) was added to the resulting solution. The organic layer was washed with $NH_4Cl_{(aq)}$ (50 mL), water (50 mL), brine (50 mL), dried, filtered and concentrated under vacuum to afford the crude sugar as a waxy yellowish solid (4.50 g). Purification by flash chromatography (100/0→50/50, v/v, PE/EtOAc) afforded pure compound **17** as a waxy solid (1.82 g, 78%). ν_{max} (liquid film) 3018, 2934, 2400, 1492, 1214, 1077, 769 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.57–7.50 (6H, m, Ar), 7.41–7.18 (22H, m, Ar), 6.93–6.90 (2H, m, Ar), 5.10 (0.35H, d, *J* 2.9 Hz, α, H-1), 5.02 (0.65H, d, *J* 11.6 Hz, C*H*$_2$–Ph), 4.77–4.63 (3.65H, m, C*H*$_2$–Ph), 4.58 (0.65H, d, *J* 6.9 Hz, β, H-1), 4.51 (0.65H, d, *J* 11.8 Hz, C*H*$_2$–Ph), 4.39–4.32 (1H, m, C*H*$_2$–Ph), 4.01 (0.35H, ddd, *J* 5.1, 9.1, 11.6 Hz, α, H-3), 3.88 (0.35H, ddd, *J* 1.5, 4.5, 9.9 Hz, α, H-6), 3.73 (0.65H, t, *J* 9.1 Hz, β, H-4), 3.67–3.57 (1.65H, m, β, H-6, β, H-5, α, H-5), 3.51 (0.35H, dd, *J* 1.7, 10.0 Hz, α, H-6′), 3.37 (0.65H, ddd, *J* 1.4, 4.2, 9.0 Hz, β, H-3), 3.29 (0.65H, dd, *J* 4.1, 9.8 Hz, β, H-6′), 3.26 (0.35H, dd, *J* 4.8, 11.7 Hz, α, H-4), 2.43–2.34 (1H, m, α and β, H-2), 1.86–1.75 (1H, m, α and β, H-2′); ^{13}C NMR ($CDCl_3$) δ 144.1, 144.0, 138.3, 138.1, 137.4, 128.9, 128.5, 128.4 (2), 128.1 (2), 127.9, 127.8, 127.7 (2), 127.5 (2), 126.9 (2), 98.5, 96.1, 86.3 (2), 79.5, 78.8, 78.3, 77.9, 75.0, 72.1, 71.7, 71.3, 70.0, 68.5, 63.0, 62.9, 37.9, 35.6; HRMS (ES) Calcd for $C_{46}H_{44}O_5Na$ (M+Na^+) 699.3086 found 699.3088.

4.1.6. Benzyl 3,4-di-*O*-benzyl-2-deoxy-D-glucopyranoside 19.[20] A solution of aluminium trichloride (80 mg, 0.60 mmol) in dry Et_2O (5 mL) was added to a solution of benzyl-6-*O*-trityl-3,4-di-*O*-benzyl-2-deoxy-D-glucopyranoside **17** (400 mg, 0.60 mmol) in dry DCM (20 mL). The reaction mixture turned bright yellow. After 25 min under stirring the reaction mixture was quenched by the addition of $NaHCO_3$ (10 mL). After dilution with Et_2O (40 mL), the organic layer was washed with water (50 mL) and brine (50 mL), then dried, filtered and concentrated under vacuum to afford the crude sugar as an orange oil (370 mg). Purification by flash chromatography (75/25→50/50, v/v, PE/EtOAc) afforded three fractions of the pure detritylated sugar **19** as a colourless oil: fraction 1: 98 mg, β-anomer; fraction 2: 49 mg, mixture of anomers and fraction 3: 40 mg, α-anomer. β-Anomer: ν_{max} (liquid film) 3464, 2922, 1491, 1365, 1211, 1059 cm^{-1}; $[\alpha]_D^{20}$ −22.1 (*c* 0.021, $CHCl_3$) (lit.[20b] $[\alpha]_D^{20}$ −26.7 (*c* 1.1, $CHCl_3$)); ^1H NMR ($CDCl_3$) δ 7.38–7.29 (15H, m, Ar), 4.94 (1H, d, *J* 11.0 Hz, C*H*$_2$–Ph), 4.86 (1H, d, *J* 11.9 Hz, C*H*$_2$–Ph), 4.69 (1H, d, *J* 12.7 Hz, C*H*$_2$–Ph), 4.68 (1H, d, *J* 11.9 Hz, C*H*$_2$–Ph), 4.67 (1H, d, *J* 11.0 Hz, C*H*$_2$–Ph), 4.67 (1H, d, *J* 11.0 Hz, C*H*$_2$–Ph), 4.60 (1H, d, *J* 12.7 Hz, C*H*$_2$–Ph), 4.58 (1H, dd, *J* 1.9, 9.5 Hz, H-1), 3.88 (1H, dd, *J* 2.8, 11.7 Hz, H-6), 3.74 (1H, dd, *J* 4.8, 11.8 Hz, H-6′), 3.68 (1H, ddd, *J* 5.0, 8.7, 11.7 Hz, H-3), 3.50 (1H, appt, *J* 9.1 Hz, H-4), 3.31 (1H, ddd, *J* 3.0, 4.8, 9.5 Hz, H-5), 2.38 (1H, ddd, *J* 1.9, 5.0, 12.6 Hz, H-2), 1.68 (1H, ddd, *J* 9.9, 11.8, 12.4 Hz, H-2′); ^{13}C NMR ($CDCl_3$) δ 138.2 (Ar), 137.3, 128.4 (Ar), 128.1 (Ar), 127.9 (Ar), 127.8 (Ar), 127.6 (Ar), 98.9 (C-1), 79.2, 78.1, 75.3, 75.0, 71.4, 70.9, 62.5, 36.7 (C-2); HRMS (ES) Calcd for $C_{27}H_{30}O_5Na$ (M+Na^+) 457.1991 found 457.1994. α-anomer: ν_{max} (liquid film) 3466, 2925, 1496, 1367, 1213, 1059 cm^{-1}; $[\alpha]_D^{20}$ +46.1 (*c* 0.015, $CHCl_3$) (lit.[20a] $[\alpha]_D^{20}$ 53 (*c* 1.5, $CHCl_3$)); ^1H NMR ($CDCl_3$) δ 7.28–7.20 (15H, m, Ar), 4.93 (1H, d, *J* 2.9 Hz, H-1), 4.87 (1H, d, *J* 11.0 Hz, C*H*$_2$–Ph), 4.62–4.55 (4H, m, C*H*$_2$–Ph), 4.36 (1H, d, *J* 11.9 Hz, C*H*$_2$–Ph), 3.99 (1H, ddd, *J* 5.0, 8.9, 11.4 Hz, H-3), 3.74 (3H, m), 3.46 (1H, t, *J* 9.3 Hz, H-4), 2.26 (1H, ddd, *J* 0.8, 4.9, 13.0 Hz, H-2), 1.61 (1H, ddd, *J* 3.7, 11.5, 13.1 Hz, H-2′); ^{13}C NMR ($CDCl_3$) δ 138.6 (Ar), 138.3, 137.5 (Ar), 128.4 (2, Ar), 128.3 (Ar), 128.0 (Ar), 127.9 (Ar), 127.8 (Ar), 127.7 (Ar), 127.6 (Ar), 96.7 (C-1), 78.2, 75.0, 71.8, 71.5, 68.9, 62.2, 35.5 (C-2); HRMS (ES) Calcd for $C_{27}H_{30}O_5Na$ (M+Na^+) 457.1991 found 457.1995.

4.1.7. Benzyl 2,3,4-tri-*O*-benzyl-5-*C*-(hydroxymethyl)-D-glucopyranoside 22α/β. To a solution of oxalyl chloride (740 μL, 8.48 mmol) in dry DCM (25 mL) cooled to −78 °C was added DMSO (1.2 mL, 16.96 mmol) diluted in dry DCM (25 mL) and the resulting solution was stirred for 40 min. To the reaction mixture was then slowly added a solution of benzyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside **18** (1.15 g, 2.12 mmol) in dry DCM (5 mL). Stirring was continued at −78 °C for 2 h. Et_3N (4.73 mL, 33.9 mmol) was added at −78 °C and after 10 min the solution was warmed up to room temperature. Water (50 mL) was then added, the aqueous layer was extracted with Et_2O (2×75 mL). The combined organic layers were washed with $NH_4Cl_{(aq)}$, brine, dried, filtered and concentrated under vacuum to afford the crude aldehyde **20α/β** (1.11 g, 100%) as a yellow oil. This crude aldehyde was co-evaporated with toluene (four times) in order to remove residual water and used without any further purification. Formaldehyde (3.1 mL of 37 wt % solution in water) and NaOH (7.0 mL of 1.0 M aqueous solution) were added to a solution of crude aldehyde (1.11 g, 2.12 mmol) in 1,4 dioxane (20 mL). The resulting reaction mixture was kept under stirring at room temperature for 48 h. After dilution with EtOAc (40 mL), the reaction mixture was neutralised with hydrochloric acid (7.0 mL of 1.0 M solution). The aqueous layer was then extracted with EtOAc (50 mL×2). Combined organic layers were then washed with water (40 mL), $NaHCO_3$ (40 mL) and brine (40 mL), dried, filtered and concentrated under reduced pressure to afford the crude diol as an orange oil. Purification by flash chromatography (75/25→50/50, v/v, PE/ EtOAc) afforded the diol **22** (0.64 g, 53%) as a colourless oil. ν_{max} (liquid film) 3445 (br), 2929, 1722, 1043 (br) cm^{-1}; $[\alpha]_D^{20}$ −22.0 (*c* 0.03, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.45–7.25 (20H, m, Ar), 5.07 (1H, d, *J* 10.8 Hz, C*H*$_2$–Ph), 4.99 (1H, d, *J* 11.0 Hz, C*H*$_2$–Ph), 4.96 (1H, d, *J* 4.0 Hz, H-1), 4.89 (1H, d, *J* 10.8 Hz, C*H*$_2$–Ph), 4.85 (1H, d, *J* 12.4 Hz, C*H*$_2$–Ph), 4.69 (1H, d, *J* 11.0 Hz, C*H*$_2$–Ph), 4.65 (1H, d, *J* 11.7 Hz, C*H*$_2$–Ph), 4.64 (1H, d, *J* 12.4 Hz, C*H*$_2$–Ph), 4.52 (1H, d, *J* 11.7 Hz, C*H*$_2$–Ph), 4.35 (1H, t, *J* 9.7 Hz, H-3), 4.16 (1H, dd, *J* 2.7, 12.0 Hz, H-6), 3.93 (1H, d, *J* 9.6 Hz, H-4), 3.80–3.67 (3H, m, H-6′, H-7, H-7′), 3.53 (1H, dd, *J* 4.0, 9.9 Hz, H-2); ^{13}C NMR ($CDCl_3$) δ 138.5 (Ar), 137.8 (Ar), 137.7 (Ar), 136.6 (Ar), 128.3 (Ar), 128.2 (Ar), 128.1 (Ar), 127.9 (Ar), 127.7 (2, Ar), 127.6 (2, Ar), 127.4 (Ar), 96.2 (C-1), 80.0 (C-2), 79.8 (C-4), 79.6 (C-5), 78.6 (C-3), 75.9 (O–*C*H$_2$–Ph), 75.4 (O–*C*H$_2$–Ph), 72.9

(O–CH_2–Ph), 70.2 (O–CH_2–Ph), 64.5 (C-6/C7), 64.3 (C-6/C-7); HRMS (ES) Calcd for $C_{35}H_{42}O_7N$ (M+NH_4^+) 588.2956 found 588.2953.

4.1.8. Benzyl 3,4-di-*O*-benzyl-5-*C*-(hydroxymethyl)-2-deoxy-D-glucopyranoside 23α/β. To a solution of oxalyl chloride (1.75 mL, 20.0 mmol) in dry DCM (20 mL) cooled to −78 °C was added DMSO (2.83 mL, 40.0 mmol) diluted in dry DCM (20 mL) and the resulting solution was stirred for 40 min. To the reaction mixture was then slowly added a solution of benzyl 3,4-di-*O*-benzyl-2-deoxy-D-glucopyranoside **19** (2.22 g, 5.0 mmol) in dry DCM (20 mL). Stirring was continued at −78 °C for 2 h. Et_3N (11.15 mL, 80.0 mmol) was added at −78 °C and after 10 min the solution was warmed up to room temperature. Water (40 mL) was then added, the aqueous layer was extracted with Et_2O (2×40 mL). The combined organic layers were washed with $NH_4Cl_{(aq)}$, brine, dried, filtered and concentrated under vacuum to afford the crude aldehyde **21** (2.26 g, 98%) as a yellow oil. This crude aldehyde was co-evaporated with toluene (four times) in order to remove residual water and used without any further purification. Formaldehyde (0.73 mL of 37 wt % solution in water) and NaOH (1.5 mL of 1.0 M aqueous solution) were added to a solution of crude aldehyde (220 mg, 0.5 mmol) in 1,4 dioxane (10 mL). The resulting reaction mixture was stirred at room temperature for 48 h. After dilution with EtOAc (30 mL), the reaction mixture was neutralised with hydrochloric acid (2.0 mL of 1.0 M solution). The aqueous layer was then extracted with EtOAc (40 mL×2). The combined organic layers were then washed with water (20 mL), $NaHCO_3$ (20 mL) and brine (20 mL), dried, filtered and concentrated under reduced pressure to afford the crude diol as a yellowish oil. Purification by flash chromatography (75/25→50/50, v/v, PE/EtOAc) afforded the diol **23** (100 mg, 43% overall) as a colourless oil 40 mg first fraction (α-anomer) and 60 mg second fraction (β-anomer). α-Anomer: ν_{max} (liquid film) 3468 (br), 3018, 2934, 1216, 1026 (br), 756 cm^{-1}; $[\alpha]_D^{20}$ +15.3 (*c* 0.018, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.36–7.26 (15H, m, Ar), 4.96 (1H, d, *J* 11.1 Hz, CH_2–Ph), 4.91 (1H, dd, *J* 1.4, 9.1 Hz, H-1), 4.83 (1H, d, *J* 11.9 Hz, CH_2–Ph), 4.83 (1H, d, *J* 11.9 Hz, CH_2–Ph), 4.68 (1H, d, *J* 11.7 Hz, CH_2–Ph), 4.65 (1H, d, *J* 11.1 Hz, CH_2–Ph), 4.60 (1H, d, *J* 11.3 Hz, CH_2–Ph), 4.59 (1H, d, *J* 11.3 Hz, CH_2–Ph), 3.98–3.93 (1H, m, H-3), 3.86–3.82 (1H, m, H-6), 3.83 (1H, d, *J* 8.2 Hz, H-4), 3.75–3.64 (3H, m, H-6′, H-7 and H-7′), 2.42–2.36 (1H, m, H-2), 1.68 (1H, m, H-2′); ^{13}C NMR ($CDCl_3$) δ 138.1 (Ar), 138.0 (Ar), 137.4 (Ar), 128.5 (Ar), 127.9 (2, Ar), 127.7 (Ar), 127.6 (Ar), 95.3 (C-1), 80.0 (C-4), 78.2 (C-3), 77.2 (C-5), 76.0 (O–CH_2–Ph), 71.5 (O–CH_2–Ph), 70.8 (O–CH_2–Ph), 65.4 (C-6), 63.3 (C-7), 36.4 (C-2); HRMS (ES) Calcd for $C_{28}H_{36}O_6N$ (M+NH_4^+) 482.2537 found 482.2541. β-Anomer: ν_{max} (liquid film) 3467 (br), 3016, 2932, 1214, 1028 (br), 755 cm^{-1}; $[\alpha]_D^{20}$ −28.0 (*c* 0.02, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.38–7.28 (15H, m, Ar), 5.04 (1H, dd, *J* 1.5, 4.0 Hz, H-1), 5.00 (1H, d, *J* 11.1 Hz, CH_2–Ph), 4.77 (1H, d, *J* 12.0 Hz, CH_2–Ph), 4.69 (1H, d, *J* 11.4 Hz, CH_2–Ph), 4.67 (1H, d, *J* 11.1 Hz, CH_2–Ph), 4.66 (1H, d, *J* 11.4 Hz, CH_2–Ph), 4.49 (1H, d, *J* 12.0 Hz, CH_2–Ph), 4.32 (1H, ddd, *J* 4.7, 9.3, 11.3 Hz, H-3), 4.10 (1H, dd, *J* 3.1, 12.0 Hz, H-6), 3.85 (1H, d, *J* 9.3 Hz, H-4), 3.77–3.68 (2H, m, H-6 and H-7), 2.99 (1H, dd, *J* 3.2, 10.1 Hz, H-7′), 2.34 (1H, ddd, *J* 1.5, 4.7, 13.2 Hz, H-2), 1.71 (1H, ddd, *J* 3.5, 4.1, 13.2 Hz, H-2′); ^{13}C NMR ($CDCl_3$) δ 138.4 (Ar), 138.0 (Ar), 128.5 (Ar), 128.4 (Ar), 127.9 (Ar), 127.7 (Ar), 127.6 (Ar), 97.4 (C-1), 80.2 (2, C-5 and C-4), 75.9 (O–CH_2–Ph), 74.1 (C-3), 71.9 (O–CH_2–Ph), 70.2 (O–CH_2–Ph), 65.0 (C-6), 64.6 (C-7), 35.7 (C-2); HRMS (ES) Calcd for $C_{28}H_{36}O_6N$ (M+NH_4^+) 482.2537 found 482.2541.

4.1.9. Benzyl 6-methylene-2,3,4-tri-*O*-benzyl-D-glucopyranoside 24. To a solution of oxalyl chloride (1.48 mL, 16.93 mmol) in dry DCM (20 mL) cooled to −78 °C was added dimethylsulfoxide (2.4 mL, 33.86 mmol) diluted in dry DCM (20 mL) and the resulting solution was stirred for 40 min. To the reaction mixture was then added slowly a solution of benzyl 2,3,4-tri-*O*-benzyl-D-glucopyranoside (2.26 g, 4.23 mmol) in dry DCM (20 mL) and stirring was continued at −78 °C for 2 h. Et_3N (9.4 mL, 67.73 mmol) was then added still at −78 °C and after 10 min reaction mixture was then allowed to warm up to room temperature. Water (30 mL) was added, the aqueous layer was extracted with diethyl ether (2×40 mL), combined organic layers were washed with $NH_4Cl_{(aq)}$, brine, dried, filtered and concentrated under vacuum to afford the crude aldehyde as a yellow oil. This crude aldehyde was co-evaporated with toluene (four times) to remove residual water and use without any further purification.

n-BuLi (5.06 mL of a 2.5 M solution in hexane, 12.65 mmol) was slowly added to a suspension of methyltriphenylphosphonium bromide (4.52 g, 12.65 mmol) in dry THF (50 mL), cooled to −78 °C. After 10 min under stirring at −78 °C the resulting orange solution was warmed up to 0 °C and stirred for 1 h. The crude aldehyde diluted in dry THF (50 mL) was then slowly added to the ylide still at 0 °C. The reaction mixture was stirred overnight and allowed to slowly warm up to room temperature. The reaction mixture was quenched by the addition of $NH_4Cl_{(aq)}$ (50 mL). The aqueous layer was extracted with Et_2O (2×40 mL). The combined organic layers were dried, filtered and concentrated under vacuum to afford the crude alkene. Purification by flash chromatography (100/0→90/10, v/v, PE/EtOAc) gave the alkene **24** (1.19 g, 53%) as a yellowish oil. β-Anomer: ν_{max} (liquid film) 3030, 2906 (br), 1951, 1874, 1809, 1725, 1605, 1216, 1068 (br), 756 cm^{-1}; $[\alpha]_D^{20}$ −23.9 (*c* 0.021, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.40–7.18 (20H, m, Ar), 5.98 (1H, ddd, *J* 5.9, 10.5, 17.1 Hz, H-6), 5.47 (1H, dt, *J* 1.5, 17.3 Hz, H-7), 5.30 (1H, dt, *J* 1.4, 10.4 Hz, H-7′), 4.97 (1H, d, *J* 11.9 Hz, CH_2–Ph), 4.96 (1H, d, *J* 10.8 Hz, CH_2–Ph), 4.90 (1H, d, *J* 10.9 Hz, CH_2–Ph), 4.80 (1H, d, *J* 10.8 Hz, CH_2–Ph), 4.77 (1H, d, *J* 10.6 Hz, CH_2–Ph), 4.73 (1H, d, *J* 10.9 Hz, CH_2–Ph), 4.68 (1H, d, *J* 11.9 Hz, CH_2–Ph), 4.62 (1H, d, *J* 10.6 Hz, CH_2–Ph), 4.57 (1H, d, *J* 7.7 Hz, H-1), 3.79 (1H, appdd, *J* 6.0, 9.6 Hz, H-5), 3.65 (1H, appt, *J* 9.1 Hz, H-4), 3.52 (1H, dd, *J* 7.8, 9.2 Hz, H-2), 3.33 (1H, appt, *J* 9.3 Hz, H-3); ^{13}C NMR ($CDCl_3$) δ 138.6 (Ar), 138.4 (Ar), 138.0 (Ar), 137.4 (Ar), 134.6 (C-6), 128.4 (2, Ar), 128.3 (2, Ar), 128.1 (Ar), 128.0 (Ar), 127.9 (2, Ar), 127.8 (Ar), 127.6 (2, Ar), 117.8 (C-7), 102.5 (C-1), 84.4 (C-4), 82.3 (C-3), 82.1 (C-2), 75.8 (*C*H_2–Ph), 75.6 (*C*H_2–Ph), 75.1 (C-5), 74.9 (*C*H_2–Ph), 71.2 (*C*H_2–Ph); $C_{35}H_{36}O_5$ Calcd C 78.33, H 6.76 found C 78.31, H 6.81.

4.1.10. Benzyl-6-methylene-3,4-di-*O*-benzyl-2-deoxy-D-glucopyranoside 25. To a solution of oxalyl chloride

(1.75 mL, 20.0 mmol) in dry DCM (20 mL) cooled at −78 °C was added DMSO (2.83 mL, 40.0 mmol) diluted in dry DCM (20 mL) and the resulting solution was stirred for 40 min. To the reaction mixture was then slowly added a solution of benzyl 3,4-di-*O*-benzyl-2-deoxy-D-glucopyranoside (2.22 g, 5.0 mmol) in dry DCM (20 mL). Stirring was continued at −78 °C for 2 h. Et_3N (11.15 mL, 80.0 mmol) was added at −78 °C and after 10 min the reaction mixture was allowed to warm up to room temperature. Water (40 mL) was then added, the aqueous layer was extracted with Et_2O (2×40 mL). The combined organic layers were washed with $NH_4Cl_{(aq)}$, brine, dried, filtered and concentrated under vacuum to afford the crude aldehyde **21** (2.26 g, 98%) as a yellow oil. This crude aldehyde was coevaporated with toluene (four times) in order to remove residual water and used without any further purification.

n-BuLi (4.0 mL of a 2.5 M solution in Hex, 10.0 mmol) was slowly added to a suspension of methyltriphenylphosphonium bromide (3.57 g, 10.0 mmol) in dry THF (20 mL), cooled to −78 °C. After 10 min under stirring at −78 °C the resulting orange solution was warmed up to 0 °C and stirred for 1 h. The crude aldehyde (0.76 g, 3.33 mmol) diluted in dry THF (20 mL) was then slowly added to the ylide solution still at 0 °C. The reaction mixture was allowed to slowly warm up to room temperature and kept under stirring overnight. The reaction mixture was quenched with $NH_4Cl_{(aq)}$ (50 mL); the aqueous layer was extracted with Et_2O (2×50 mL), combined organic layers were washed with NH_4Cl (50 mL), water (50 mL) and brine (50 mL), dried, filtered and concentrated under reduced pressure to afford the crude alkene. Purification by flash chromatography (75/25, v/v, PE/EtOAc) yielded the alkene **25** (0.63 g, 44%) as a yellowish oil. ν_{max} (liquid film) 3033, 2912 (br), 1955, 1878, 1809, 1723, 1605, 1219, 1058 (br), 756 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.35–7.25 (15H, m, Ar), 6.00 (1H, ddd, *J* 6.3, 10.5, 17.0 Hz, H-6), 5.42 (1H, dt, *J* 1.5, 10.5 Hz, H-7), 5.27 (1H, dt, *J* 1.3, 17.3 Hz, H-7′), 5.02 (1H, d, *J* 2.9 Hz, H-1), 4.84 (1H, d, *J* 10.7 Hz, C*H*$_2$–Ph), 4.67–4.64 (3H, m, C*H*$_2$–Ph×3), 4.44 (1H, d, *J* 11.9 Hz, C*H*$_2$–Ph), 4.16 (1H, dd, *J* 6.5, 9.6 Hz, H-5), 4.04 (1H, ddd, *J* 5.1, 8.8, 11.4 Hz, H-3), 3.27 (1H, appt, *J* 9.2 Hz, H-4), 2.33 (1H, ddd, *J* 1.2, 5.1, 13.0 Hz, H-2), 1.73 (1H, ddd, *J* 3.7, 11.5, 13.0 Hz, H-2′); ^{13}C NMR ($CDCl_3$) δ 139.4 (Ar), 138.7(Ar), 137.6 (Ar), 135.7 (C-6), 128.3 (2) (Ar), 128.0 (Ar), 127.9 (Ar), 127.6 (Ar), 127.5(Ar), 117.7 (C-7), 96.5 (C-1), 82.8 (C-4), 77.0 (O–*C*H$_2$–Ph), 75.1 (O–*C*H$_2$–Ph), 72.2 (C-5), 72.1 (C-3), 68.8 (O–*C*H$_2$–Ph), 35.7 (C-2); Calcd C 78.11, H 7.02 found C 78.06, H 7.06; HRMS (ES) Calcd for $C_{28}H_{34}O_4N$ (M+NH_4^+) 448.2482 found 448.2468.

4.1.11. Benzyl 2,3,4-tri-*O*-benzyl-*glycero*-α-D-*gluco*-heptopyranoside 26. A small crystal of osmium tetroxide was added to a solution of alkene **24** (240 mg, 0.45 mmol) and *N*-methylmorpholine *N*-oxide (121 mg, 0.90 mmol) in DCM (20 mL). The resulting solution was kept stirring overnight. The reaction mixture was then diluted with DCM (20 mL) and sodium metabisulfite (20 mL of a 10% solution) was added. The resulting biphasic solution was then stirred for 10 min. The aqueous layer was then extracted with DCM (25 mL×3). The combined organic layers were then washed with sodium metabisulfite (20 mL of 10% solution×2), water (20 mL×2) and brine (20 mL), then dried, filtered and concentrated under reduced pressure to afford the crude diol **26** as a colourless oil 285 mg, 100%), which turned into a waxy solid upon standing overnight. ν_{max} (liquid film) 3468, 3017, 2880 (br), 1454, 1216, 1068 (br), 756 cm^{-1}; $[\alpha]_D^{20}$ +2.36 (*c* 0.023, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.40–7.26 (20H, m), 5.06 (1H, d, *J* 10.8 Hz, C*H*$_2$–Ph), 5.01 (1H, d, *J* 10.9 Hz, C*H*$_2$–Ph), 4.80 (1H, d, *J* 10.8 Hz, C*H*$_2$–Ph), 4.77 (1H, d, *J* 3.9 Hz, H-1), 4.75 (1H, d, *J* 10.9 Hz, C*H*$_2$–Ph), 4.66 (1H, d, *J* 12.0 Hz, C*H*$_2$–Ph), 4.64 (1H, d, *J* 11.9 Hz, C*H*$_2$–Ph), 4.54 (1H, d, *J* 11.9 Hz, C*H*$_2$–Ph), 4.53 (1H, d, *J* 12.0 Hz, C*H*$_2$–Ph), 4.10 (1H, t, *J* 9.6, H-3), 3.86 (1H, dd, *J* 5.4, 9.7 Hz, H-5), 3.80 (1H, dd, *J* 4.4, 9.3 Hz, H-6), 3.66 (1H, dd, *J* 4.4, 11.6 Hz, H-7), 3.58 (1H, dd, *J* 4.1, 7.4 Hz, H-7′), 3.55 (1H, t, *J* 9.6 Hz, H-4), 3.50 (1H, dd, *J* 3.7, 9.6 Hz, H-2); ^{13}C NMR ($CDCl_3$) δ 138.4, 137.9, 137.3, 136.8, 128.7, 128.5, 128.4, 128.2, 128.0, 127.9, 127.7, 94.7 (C-1), 82.2 (C-3), 80.1 (C-5 or C-2), 80.0 (C-5 or C-2), 75.6 (*C*H$_2$–Ph), 75.0 (*C*H$_2$–Ph), 72.9 (*C*H$_2$–Ph), 72.7 (C-6), 70.1 (C-7), 69.1 (*C*H$_2$–Ph), 62.9 (C-4); Calcd C 73.66, H 6.71 found C 73.75, H 6.87; LRMS (ES) (M+Na^+) Calcd 593.0 found 593.1; HRMS (ES) Calcd for $C_{35}H_{42}O_7N$ (M+NH_4^+) 588.2956 found 588.2951.

4.1.12. Benzyl 3,4-di-*O*-benzyl-*glycero*-α-D-2-deoxy-*gluco*-heptopyranoside 27. A small crystal of osmium tetroxide was added to a solution of alkene **25** (200 mg, 0.46 mmol) and *N*-methylmorpholine *N*-oxide (126 mg, 0.93 mmol) in DCM (20 mL). The resulting solution was stirred overnight. The reaction mixture was then diluted with DCM (20 mL) and sodium metabisulfite (20 mL of a 10% solution) was added. The resulting biphasic solution was then kept under stirring for 10 min. The aqueous layer was then extracted with DCM (25 mL×3). The combined organic layers were then washed with sodium metabisulfite (20 mL of 10% solution×2), water (20 mL×2) and brine (20 mL), then dried, filtered and concentrated under reduced pressure to afford the crude diol **27** as a colourless oil (200 mg, 98%), which turned into a waxy solid upon standing overnight. ν_{max} (liquid film) 3465, 3018, 2875 (br), 1458, 1216, 1063 (br), 758 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.29–7.21 (15H, m, Ar), 4.99 (0.75H, d, *J* 11.0 Hz), 4.97 (0.25H, d, *J* 11.0 Hz), 4.90 (0.75H, d, *J* 3.1 Hz), 4.73 (0.25H, d, *J* 11.9 Hz), 4.64 (0.75H, d, *J* 11.6 Hz), 4.62 (0.75H, d, *J* 11.0 Hz), 4.59 (0.75H, d, *J* 11.4 Hz), 4.51 (0.75H, d, *J* 11.4 Hz), 4.50–4.38 (1.25H, m), 4.35 (1H, d, *J* 11.8 Hz), 4.05–4.00 (1H, m), 3.85–3.74 (2.25H, m), 3.65 (0.75H, dd, *J* 4.6, 11.6 Hz), 3.69–3.62 (0.25H, m), 3.57 (0.75H, dd, *J* 3.8, 11.6 Hz), 3.58–3.54 (0.25H, m), 3.48 (0.75H, appt, *J* 9.1 Hz), 3.46 (0.25H, appt, *J* 8.9 Hz), 3.30 (0.25H, dd, *J* 5.4, 9.6 Hz), 3.13 (0.75H, m), 2.32 (0.25H, ddd, *J* 1.8, 5.0, 13.0 Hz), 2.28 (0.75H, dd, *J* 5.0, 13.1 Hz), 1.60 (1H, app ddd, *J* 3.5, 11.4, 12.9 Hz); ^{13}C NMR ($CDCl_3$) δ 138.2, 137.9, 137.6, 137.5, 137.3, 137.2, 128.6, 128.5, 128.4 (2), 128.3 (2), 128.1 (2), 128.0, 127.9 (2), 127.8 (2), 127.7 (2), 127.6, 99.1, 96.1, 80.7, 80.2, 79.6, 77.9, 74.9 (2), 74.8, 72.9, 72.4, 71.4, 71.2, 70.9, 70.6, 68.9, 63.8, 63.0, 62.8, 61.2, 54.1, 53.9, 36.5, 35.2; HRMS (ES) Calcd for $C_{28}H_{36}O_6N$ (M+NH_4^+) 482.2537 found 482.2551.

4.1.13. Benzyloxydichlorophosphine. To a solution of phosphorus trichloride (6.1 mL, 70 mmol) in dry CH_3CN (25 mL) was added benzyl alcohol (1.0 mL, 10 mmol)

diluted in dry CH_3CN (25 mL). The resulting reaction solution was stirred for 1 h. The crude reaction mixture was concentrated under reduced pressure to afford the crude benzyloxydichlorophosphine as a colourless oil (2.05 g, 98%). ^{1}H NMR ($CDCl_3$) δ 7.39 (5H, br), 5.25 (2H, d, J 8.0 Hz); ^{13}C NMR ($CDCl_3$) δ 134.8 (d, J_{POCC} 7.6 Hz), 126.7, 126.3, 125.7, 63.2 (d, J_{POC} 5.4 Hz), 43.4 (d, J_{POC} 6.3 Hz), 21.1 (d, J_{PNCC} 2.0 Hz); ^{31}P ($CDCl_3$) δ 178.5.

4.1.14. (3*R*,4*S*,5*S*)-2,3,4,5,9-Pentakis-benzyloxy-1,8,10-trioxa-9-phospha-spiro[5.5]undecane 9-oxide 28. To a solution of diol **22** (200 mg, 0.35 mmol) in dry DCM (5 mL) under stirring was added Et_3N (195 μL, 1.4 mmol). The resulting solution was stirred for 5 min before the addition of benzyloxydichlorophosphine (80 mg, 0.38 mmol; freshly prepared) diluted in dry DCM (5 mL). The resulting reaction mixture was then kept stirring until TLC indicated the complete conversion of the starting material into a more lipophilic material (75/25, EtOAc/PE, v/v). The reaction mixture was then concentrated under reduced pressure to afford the crude cyclic phosphite as a colourless oil. The resulting residue was passed through a short silica pad (75/25, EtOAc/PE, v/v) to afford the pure cyclic phosphite as a colourless oil. The purity of the cyclic phosphite was checked by ^{31}P NMR (mixture of diastereoisomers). The cyclic phosphite was then rediluted in dry DCM (10 mL), and *tert*-butylhydroperoxide (230 μL of a 3.0 M solution in toluene, 0.70 mmol) was added. The resulting reaction mixture was then kept under stirring for 30 min. The solution was then concentrated under reduced pressure to afford the crude cyclic phosphate triester as a yellowish oil. The residue was then purified through a short silica pad (75/25 → 0/100, v/v, PE/EtOAc) to afford the pure cyclic phosphate triester **28** as colourless oil (180 mg, 71%). ^{1}H NMR ($CDCl_3$) δ 7.45–7.24 (25H, m, Ar), 5.20–4.23 (14H, m), 4.06–3.32 (4H, m); ^{13}C NMR ($CDCl_3$) δ 137.8, 137.6, 136.9, 136.8, 136.7, 133.0, 129.9, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0 (2), 127.9 (2), 127.8, 127.7, 127.3, 126.8, 98.1, 97.3, 82.2, 81.5, 81.4, 80.7, 78.0, 75.5, 75.4, 75.0, 74.7, 74.5, 74.1, 73.9 (d, J_{POCC} 7.3 Hz), 73.3 (d, J_{POCC} 6.6 Hz), 72.8 (d, J_{POC} 6.2 Hz), 71.9 (d, J_{POC} 6.2 Hz), 71.0, 70.9, 70.1 (d, J_{POC} 5.4 Hz), 68.9 (d, J_{POC} 5.1 Hz), 66.9 (d, J_{POC} 5.4 Hz), 66.3 (d, J_{POC} 6.7 Hz); ^{31}P NMR ($CDCl_3$) δ −4.3, −7.3; HRMS (FAB^-) Calcd for $C_{42}H_{43}O_9P$ (M^+) 722.2723 found 722.2702.

4.1.15. (4*R*,5*S*)-2,4,5,9-Tetrakis-benzyloxy-1,8,10-trioxa-9-phospha-spiro[5.5]undecane 9-oxide 29. To a stirred solution of diol **23** (55 mg, 0.108 mmol) in dry DCM (5 mL) was added Et_3N (60 μL, 0.43 mmol). The resulting solution was stirred for 5 min before the addition of benzyloxydichlorophosphine (25 mg, 0.12 mmol; freshly prepared) diluted in dry DCM (5 mL). The resulting reaction mixture was then kept stirring until TLC indicated the complete conversion of the starting material into a more lipophilic material (75/25, EtOAc/PE, v/v). The reaction mixture was then concentrated under reduced pressure to afford the crude cyclic phosphite as a colourless oil. The resulting residue was passed through a short silica column (75/25, EtOAc/PE, v/v) to afford the pure cyclic phosphite as a colourless oil. The purity of the cyclic phosphite was checked by ^{31}P NMR (mixture of diastereoisomers). The cyclic phosphite was then rediluted in dry DCM (10 mL), and *tert*-butylhydroperoxide (74 μL of a 3.0 M solution in toluene, 0.22 mmol) was added. The resulting reaction mixture was then kept under stirring for 30 min. The solution was then concentrated under reduced pressure to afford the crude cyclic phosphate triester as a yellowish oil. The residue was then purified through a short silica column (75/25 → 0/100, v/v, PE/EtOAc) to afford the pure cyclic phosphate triester **29** as colourless oil (54 mg, 71%). ^{1}H NMR ($CDCl_3$) δ 7.45–7.29 (20H, m, Ar), 5.20–4.23 (13H, m), 2.45 (1H, m), 1.93–1.78 (1H, m); ^{13}C NMR ($CDCl_3$) δ 137.9, 137.6, 137.4, 137.3, 137.1 (2), 135.6, 128.6 (2), 128.5, 128.4 (2), 128.3, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 97.0, 95.9, 79.0, 75.4, 74.9, 74.7, 74.5 (d, J_{POCC} 7.6 Hz), 73.9, 73.8, 73.7, 72.6 (d, J_{POCC} 6.5 Hz), 71.7, 71.6 (d, J_{POC} 7.3 Hz), 71.3 (d, J_{POC} 6.4 Hz), 70.3, 69.9 (d, J_{POC} 5.7 Hz), 68.7 (d, J_{POC} 5.2 Hz), 35.5, 33.8; ^{31}P NMR ($CDCl_3$) δ −4.0, −7.1; HRMS (FAB^-) Calcd for $C_{35}H_{37}O_8P$ (M^+) 617.2304 found 617.2301.

4.1.16. (3*R*,4*S*,5*S*,6*S*)-2,3,4,5-Tetrakis-benzyloxy-6-(2-benzyloxy-2-oxo-2-λ-5-[1,3,2]dioxaphospholan-4-yl)-tetrahydropyran 30. 2,4-Dinitrophenol (26 mg, 0.140 mmol) diluted in dry DCM (5 mL) was added to a solution of diol **26** (54 mg, 0.093 mmol) and benzyloxy-bis(diisopropylamino)phosphine (34 mg, 0.102 mmol) in dry DCM (5 mL), under stirring. The resulting reaction mixture was kept under stirring until only one peak corresponding to the cyclic phosphite was observed by ^{31}P NMR. The reaction mixture was cooled down to −78 °C prior to the addition of *tert*-butylhydroperoxide (37 μL of 5.0 M solution in decane, 0.186 mmol). After 10 min stirring at −78 °C, the reaction was allowed to warm up to room temperature and sodium thiosulfate (5 mL of 10% aqueous solution) was added. The reaction mixture was then diluted with Et_2O (20 mL). The organic layer was washed with sodium thiosulfate (15 mL), water (15 mL×2), dried, filtered and concentrated under reduced pressure to afford the crude cyclic phosphate triester **30** as a yellow oil. Purification by reversed-phase chromatography (C18-RP Supelcosyl HPLC column; CH_3CN/water) gave the pure cyclic phosphate triester (31 mg, 46%) as a colourless oil. ^{1}H NMR ($CDCl_3$) δ 7.38–7.26 (25H, m, Ar), 5.12 (2H, d, J_{POC} 4.9 Hz, CH_2–Ph×2), 5.04 (1H, d, J 10.9 Hz, CH_2–Ph), 5.00 (1H, d, J 8.1 Hz, H-1), 4.84 (1H, d, J 12.0 Hz, CH_2–Ph), 4.81 (1H, d, J 11.0 Hz, CH_2–Ph), 4.74 (1H, d, J 12.1 Hz, CH_2–Ph), 4.70 (1H, s, CH_2–Ph), 4.61 (1H, d, J 13.6 Hz, CH_2–Ph), 4.70–4.50 (1H, m), 4.58 (1H, d, J 11.7 Hz, CH_2–Ph), 4.50 (1H, d, J 11.9 Hz, CH_2–Ph), 4.12 (1H, dd, J 7.2, 14.3 Hz, H-4), 4.07 (1H, dd, J 8.7, 9.4 Hz, H-2), 3.98 (1H, dd, J 1.7, 10.5 Hz, H-7), 3.50 (1H, ddd, J 7.6, 8.0, 15.6 Hz, H-5), 3.47 (1H, dd, J 3.7, 9.6 Hz, H-3), 3.20 (1H, dd, J 8.6, 10.4 Hz, H-7′); ^{13}C NMR ($CDCl_3$) δ 138.5, 137.8, 137.3, 136.7, 135.8, 129.0, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3, 128.0, 127.9, 127.8, 127.7, 127.6, 127.0, 94.2, 82.4, 79.7, 76.3, 75.8, 75.5, 74.4, 72.7, 70.1, 69.5, 69.3, 68.8; ^{31}P NMR ($CDCl_3$) δ 17.4; HRMS (FAB^-) Calcd for $C_{42}H_{44}O_9P$ ($M+H^+$) 723.2723 found 723.2702.

4.1.17. (2*S*,3*S*,4*R*)-3,4,6-Tris-benzyloxy-2-(2-benzyloxy-2-oxo-2-λ-5-[1,3,2]dioxaphospholan-4-yl)-tetrahydropyran 31. To a solution of diol **27** (50 mg, 0.12 mmol) in dry DCM (5 mL) under stirring was added Et_3N (75 μL, 0.48 mmol). The resulting solution was stirred for 5 min before the addition of benzyloxydichlorophosphine (28 mg, 0.13 mmol; freshly prepared) diluted in dry DCM (5 mL).

The resulting reaction mixture was then stirred until TLC indicated the complete conversion of the starting material into a more lipophilic material (75/25, EtOAc/PE, v/v). The reaction mixture was then concentrated under reduced pressure to afford the crude cyclic phosphite as a colourless oil. The resulting residue was passed through a short silica column (75/25, EtOAc/PE, v/v) to afford the pure cyclic phosphite as a colourless oil. The purity of the cyclic phosphite was checked by ^{31}P NMR (mixture of diastereoisomers). The cyclic phosphite was then rediluted in dry DCM (10 mL), and *tert*-butylhydroperoxide (74 μL of a 3.0 M solution in toluene, 0.22 mmol) was added. The resulting reaction mixture was then kept stirring for 30 min. The solution was then concentrated under reduced pressure to afford the crude cyclic phosphate triester as a yellowish oil. The residue was then purified through a short silica column (75/25→0/100, v/v, PE/EtOAc) to afford the pure cyclic phosphate triester **31** as colourless oil (54 mg, 71%). ^{1}H NMR ($CDCl_3$) δ 7.45–7.29 (20H, m, Ar), 5.17–4.98 (10H, m), 4.23–4.17 (1H, m), 4.10–3.98 (2H,m), 3.60–3.54 (1H, m), 3.20–3.07 (1H), 2.36–2.30 (1H, m), 1.68–1.59 (1H, m); ^{13}C NMR ($CDCl_3$) δ 138.3, 138.2, 137.5, 137.3 (2), 137.0, 128.8 (2), 128.7, 128.6, 128.5, 128.4, 128.2, 128.0, 127.8 (2), 127.7, 127.6, 96.0, 95.8, 78.0, 77.9, 75.8, 75.7, 74.3 (2), 71.7 (d, J_{POCC} 7.7 Hz), 70.3 (d, J_{POCC} 5.5 Hz), 70.1 (d, J_{POC} 6.0 Hz), 70.0 (d, J_{POC} 5.5 Hz), 69.5 (d, J_{POC} 5.9 Hz), 68.8, 65.0, 64.9, 35.1, 35.0; ^{31}P NMR ($CDCl_3$) δ 18.23, 18.8; HRMS (ES) Calcd for $C_{35}H_{38}O_8P$ (M+H$^+$) 617.2304 found 617.2310.

4.1.18. Benzyl 6-deoxy-2,3,4-tri-*O*-benzyl-D-*gluco*-heptopyranoside. To a solution of alkene **24** (536 mg, 1 mmol) in dry THF (10 mL), was added a solution of 9-BBN (10 mL of 0.5 M solution in THF, 5 mmol). The resulting solution was heated under reflux for 5 h. The reaction mixture was then allowed to cool down to room temperature, and then cooled down to 0 °C. NaOH (12 mL of a 10% solution, 30 mmol) and followed by H_2O_2 (12 mL of a 30% solution, 105 mmol) were added slowly (exothermic reaction). The resulting biphasic reaction mixture was stirred for 30 min, then diluted with $CHCl_3$ (50 mL). The aqueous layer was then extracted with $CHCl_3$ (25 mL×2). Combined organic layers were washed with sodium metabisulfite solution (40 mL of 10% solution), water (40 mL), and brine (40 mL), then dried, filtered and concentrated under reduced pressure to afford the crude heptose as a yellowish oil (950 mg). Purification by flash chromatography (85/15→60/40, v/v, PE/EtOAc) afforded the alcohol (505 mg, 91%) as a colourless oil. ν_{max} (liquid film) 3465, 3012, 2880 (br), 1454, 1214, 1068 (br), 756 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.40–7.23 (20, m, Ar), 4.99 (1H, d, J 10.8 Hz, CH_2–Ph), 4.90 (1H, d, J 11.0 Hz, CH_2–Ph), 4.80 (1H, d, J 10.8 Hz), 4.75 (1H, d, J 3.7 Hz, H-1), 4.70 (1H, d, J 12.3 Hz), 4.66 (1H, d, J 11.9 Hz, CH_2–Ph), 4.59 (1H, d, J 11.0 Hz, CH_2–Ph), 4.54 (1H, d, J 11.9 Hz, CH_2–Ph), 4.51 (1H, d, J 12.3 Hz, CH_2–Ph), 4.02 (1H, appt, J 9.2 Hz, H-3), 3.90 (1H, dt, J 2.9, 9.5 Hz, H-5), 3.71–3.65 (2H, m, H-7, H-7′), 3.50 (1H, dd, J 3.7, 9.5 Hz, H-2), 3.25 (1H, appt, J 9.3 Hz, H-4), 2.07–2.00 (1H, m, H6), 1.66–1.60 (1H, m, H-6′); ^{13}C NMR ($CDCl_3$) δ 138.7 (Ar), 138.1(Ar), 136.9 (Ar), 128.5 (Ar), 128.4 (Ar), 128.3 (Ar), 128.0 (Ar), 127.9 (2, Ar), 127.8 (2, Ar), 127.6 (Ar), 95.1 (C-1), 81.9 (C-3), 81.7 (C-4), 80.0 (C-2), 75.7 (O–CH$_2$–Ph), 75.3 (O–CH$_2$–Ph), 73.0 (O–CH$_2$–Ph), 70.5 (O–CH$_2$–Ph), 69.1 (C-5), 60.9 (C-6), 33.9 (C-7); HRMS (FAB$^+$) Calcd for $C_{35}H_{38}O_6$ (M$^+$) 554.2668 found 554.2669

4.1.19. Phosphoric acid dibenzyl ester 2-((2*R*,3*R*,4*S*,5*R*)-3,4,5,6-tetrakis-benzyloxy-tetrahydro-pyran-2-yl)-ethyl ester (benzyl 6-deoxy-2,3,4-tri-*O*-benzyl-D-*gluco*-heptopyranoside 7-dibenzylphosphate) 36. *t*-BuLi (423 μL of a 1.7 M solution in hexanes, 0.72 mmol) was added to a solution of benzyl 6-deoxy-2,3,4-tri-*O*-benzyl-D-*gluco*-heptopyranoside (200 mg, 0.36 mmol) diluted in THF (15 mL) cooled down at −78 °C. The resulting solution was stirred at −78 °C for 10 min. A solution of TBBP (554 mg, 0.72 mmol) in THF (10 mL) was then added to the reaction mixture at −78 °C. The reaction mixture was stirred for 1 h at −78 °C, before being allowed to warm up to room temperature. After 3 h under stirring at room temperature, the reaction mixture was then diluted with $CHCl_3$ (30 mL) and $NH_4Cl_{(aq)}$ (30 mL) was then slowly added. The aqueous layer was extracted with $CHCl_3$ (30 mL×2). The combined organic layers were then washed with $NH_4Cl_{(aq)}$ (50 mL), water (50 mL) and brine (50 mL), dried, filtered and concentrated under reduced pressure to afford the crude phosphorylated sugar. Purification by flash chromatography (75/25→55/45, v/v, PE/EtOAc) afforded pure **36** as a colourless oil (138 mg, 47%). ^{1}H NMR ($CDCl_3$) δ 7.52–7.34 (30H, m, Ar), 5.18 (0.3, d, J 7.9 Hz), 5.12 (0.7H, d, J 10.5 Hz), 5.11–5.07 (0.6H, m), 5.06 (1H, d, J 12.0 Hz, CH_2–Ph), 4.99 (1H, d, J 11.4 Hz, CH_2–Ph), 4.92 (0.7H, d, J 10.8 Hz, CH_2–Ph), 4.89 (0.7H, d, J 10.9 Hz, CH_2–Ph), 4.86 (0.7H, d, J 3.6 Hz), 4.83 (0.7H, d, J 10.8 Hz, CH_2–Ph), 4.81–4.51 (6.3H, m), 4.14 (0.7H, appt, J 9.3 Hz), 4.02 (0.3H, dt, J 2.8, 9.6 Hz), 3.87 (1H, appt, J 5.8 Hz), 3.81–3.79 (1H, m), 3.74 (0.3H, appt, J 9.0 Hz), 3.44 (0.3H, appt, J 9.2 Hz), 3.36 (0.7H, appt, J 9.3 Hz), 1.86–1.72 (2H, m); ^{13}C NMR ($CDCl_3$) δ 138.7 (Ar), 138.4 (Ar), 138.3 (Ar), 138.1 (Ar), 138.0 (2, Ar), 137.9 (Ar), 137.1 (Ar), 136.8 (Ar), 135.8 (2, Ar), 128.7 (Ar), 128.5 (Ar), 128.4 (2, Ar), 128.1 (2, Ar), 128.0 (2, Ar), 127.9 (Ar), 127.8 (3, Ar), 127.7 (Ar), 127.6 (2, Ar), 102.7, 94.9, 84.6, 82.3, 81.8, 81.6, 81.3, 80.0, 75.7, 75.3, 75.2, 74.9, 73.9, 73.0, 71.4, 70.4, 69.2, 69.0, 60.8, 60.3, 34.0, 33.9; ^{31}P NMR ($CDCl_3$) δ 0.6, 0.5. MS (ES) Calcd for $C_{49}H_{51}O_9P$ (M+Na$^+$) 838; (M−261$^+$)(phosphate) 577.

4.1.20. 2-Benzyloxy-2-((1*S*,2*R*,3*S*)-1,2,3,4-tetrakis-benzyloxy-butyl)-propane-1,3-diol 32. An extensive synthetic sequence starting from D-glucose was developed to access this glucitol derivative. However, only the full characterisation of this compound is provided here. ^{1}H NMR ($CDCl_3$) δ 7.23–7.14 (25H, m, Ar), 4.66 (1H, d, J 11.1, CH_2–Ph), 4.64 (1H, d, J 11.6, CH$_2$–Ph), 4.58 (2H, s, H-6/H-6′ or H-7/H-7′), 4.55 (1H, d, J 11.3, CH_2–Ph), 4.53 (1H, d, J 11.4, CH_2–Ph), 4.49 (2H, d, J 11.8, CH_2–Ph), 4.28 (2H, s, H-6/H-6′ or H-7/H-7′), 3.95 (1H, appt, J 4.5, H-3), 3.89 (1H, d, J 4.2, H-4), 3.82 (1H, appdd, J 4.9, 10.5, H-2), 3.80 (1H, d, J 12.3, CH_2–Ph), 3.76 (1H, d, J 12.4, CH_2–Ph), 3.70 (1H, d, J 12.4, CH_2–Ph), 3.68 (1H, d, J 12.1, CH_2–Ph), 3.59 (1H, dd, J 4.2, 10.3, H-1), 3.50 (1H, dd, J 5.7, 10.2, H-1′); ^{13}C NMR ($CDCl_3$) δ 138.6 (Ar), 138.4 (Ar), 138.4 (Ar), 138.1 (Ar), 137.9, 128.3 (2, Ar), 127.9 (Ar), 127.6 (2, Ar), 127.5 (Ar), 80.8, 79.4, 78.5, 76.6, 74.9, 74.0, 73.1, 73.0, 70.6, 64.8, 62.8, 62.3; LRMS (FAB) Calcd for $C_{42}H_{46}O_7$ (M+Na$^+$) 685.0 found 685.0.

4.1.21. Benzyl 6-dihydroxymethylene-1,2,3,4,5-penta-*O*-benzyl-D-glucitol 33. An extensive synthetic sequence from D-glucose was developed to access this glucitol derivative. However, only the full characterisation of this compound is provided here. ν_{max} (liquid film) 3463, 3013, 2880 (br), 1454, 1217, 1063 (br), 756 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.33–7.25 (23H, m, Ar), 7.20–7.18 (2H, m, Ar), 4.76 (1H, d, *J* 11.6 Hz, C*H*$_2$–Ph), 4.75 (1H, d, *J* 11.3 Hz, C*H*$_2$–Ph), 4.71 (1H, d, *J* 11.3 Hz, C*H*$_2$–Ph), 4.66 (1H, d, *J* 11.3 Hz, C*H*$_2$–Ph), 4.65 (1H, d, *J* 11.3 Hz, C*H*$_2$–Ph), 4.56 (1H, d, *J* 11.6 Hz, C*H*$_2$–Ph), 4.55 (1H, d, *J* 11.5 Hz, C*H*$_2$–Ph), 4.51 (2H, d, *J* 2.4, 12.0 Hz, C*H*$_2$–Ph), 4.37 (1H, d, *J* 11.5 Hz, C*H*$_2$–Ph), 4.08 (1H, dd, *J* 2.6, 5.8 Hz, H-7), 3.90–3.84 (3H, m, H-3, H-4 and H-5), 3.75 (1H, dd, *J* 3.8, 11.3 Hz, H-2), 3.69 (1H, dd, *J* 2.6, 7.2 Hz, H-7′), 3.67–3.63 (2H,m, H-1′ and H-6), 3.57 (1H, dd, *J* 5.5, 10.3 Hz, H-1); ^{13}C NMR ($CDCl_3$) δ 138.2 (2), 138.1 (2), 138.0, 128.4, 128.3 (2), 128.1, 128.0, 127.8, 127.7 (2), 81.1, 80.0, 79.6, 78.5, 7.8, 74.6, 73.3, 72.9, 72.7, 71.1, 69.9, 63.9; HRMS (ES) Calcd for $C_{42}H_{47}O_7$ (M+H^+) 663.3316 found 663.3324.

4.1.22. (3*R*,4*S*,5*S*)-9-Oxo-1,8,10-trioxa-9-λ-5-phospha-spiro[5.5]undecane-2,3,4,5,9-pentaol 9. A suspension of Pd/C (50 mg, 10% Pd/C), potassium carbonate (5.5 mg, 38.7 μmol) and cyclic phosphate triester **28** (55 mg, 77.5 μmol) in MeOH (10 mL) was stirred overnight under a positive pressure of H_2 (balloon). The reaction mixture was then filtered and concentrated under reduced pressure. ^{31}P and ^1H NMR spectroscopic analyses (D_2O) showed partial deprotection. The crude reaction mixture was resuspended in MeOH (10 mL). More catalyst (50 mg, Pd/C 10%) and K_2CO_3 were added and the resulting reaction mixture was then stirred overnight under a positive pressure of H_2 (balloon). This cycle was repeated until NMR analysis showed complete removal of the benzyl protecting group. Three cycles were needed to fully deprotect **28**. The crude **9** was then filtrated and lyophilised to afford **9** as an hydroscopic powder (21.0 mg, 98%). ^1H NMR (D_2O) δ 4.76–4.73 (1H, m), 4.40–4.25 (4H, m), 3.82–3.70 (1H, m), 3.41–3.34 (1H, m), 3.19–3.06 (1H, m); ^{13}C NMR (D_2O) δ 93.2, 92.1, 75.8, 75.0, 74.1, 74.0, 72.8, 72.7, 72.1, 71.7, 69.3 (2), 64.1 (2); ^{31}P NMR (D_2O) δ −0.9 (s), −2.2 (s); LRMS (ES) Calcd for $C_7H_{13}O_9P$ (M−1) 271.1 found 271.0; HRMS (ES) Calcd for $C_7H_{12}O_9P$ (M−H^-) 271.0219 found 271.0215.

4.1.23. (4*R*,5*S*)-9-Oxo-1,8,10-trioxa-9-λ-5-phospha-spiro-[5.5]undecane-2,4,5,9-tetraol 10. A suspension of Pd/C (50 mg, 10% Pd/C), potassium carbonate (5.5 mg, 38.7 μmol) and cyclic phosphate triester **29** (54 mg, 87.7 μmol) in MeOH (10 mL) was stirred overnight under a positive pressure of H_2 (balloon). The reaction mixture was then filtered and concentrated under reduced pressure. ^{31}P and ^1H NMR spectroscopic analysis (D_2O) showed partial deprotection. The crude reaction mixture was resuspended in MeOH (10 mL). More catalyst (50 mg, Pd/C 10%) and K_2CO_3 were added and the resulting reaction mixture was then stirred overnight under a positive pressure of H_2 (balloon). This cycle was repeated until NMR analysis showed the complete removal of the benzyl protecting group. Two cycles were needed to fully deprotect **29**. The crude reaction mixture was then filtrated and lyophilised to afford **10** as a hygroscopic powder (22.1 mg, 98%). ^1H NMR (D_2O) δ 5.20 (0.6H, dd, *J* 4.6, 4.8), 5.04 (0.4H, d, *J* 5.4), 4.50–3.81 (4H, m), 3.21–3.02 (2H, m), 2.16–1.97 (2H, m); ^{13}C NMR (D_2O) δ 106.2, 105.6, 82.9, 80.2, 72.8, 72.6, 71.8, 71.5, 71.4, 56.5, 56.4, 50.2, 43.4, 42.4; ^{31}P NMR (D_2O) δ −1.8 (br s); LRMS (ES) Calcd for $C_7H_{13}O_8P$ (M−1) 255.1 found 255.0; HRMS (ES) Calcd for $C_7H_{12}O_8P$ (M−H^-) 255.0270 found 255.0265.

4.1.24. (3*R*,4*S*,5*S*,6*S*)-6-(2-Hydroxy-2-oxo-2-λ-5-[1,3,2]-dioxaphospholan-4-yl)-tetrahydro-pyran-2,3,4,5-tetraol 13. Sodium metal (100 mg) was added to a solution of cyclic phosphate triester **30** (60 mg, 0.083 mmol) in liquid ammonia. The resulting reaction mixture was stirred at −78 °C for 2 h. MeOH (10 mL) and NH_4Cl (350 mg) were then sequentially added. The resulting reaction mixture was warmed up to room temperature overnight to allow evaporation of the ammonia. The crude reaction mixture was then concentrated under reduced pressure to afford the crude cyclic phosphate diester **13** (mixture of α and β-anomers) (450 mg, heavily contaminated with salts). Attempts to desalt the title compound proved unsuccessful, hence the lack of ^1H NMR data. ^{31}P NMR (D_2O) δ 19.4, 19.3; LRMS (ES) Calcd for $C_7H_{13}O_9P$ (M−1) 271.1 found 271.0; HRMS (ES) Calcd for $C_7H_{12}O_9P$ (M−H^-) 271.0219 found 271.0213.

4.1.25. (1*S*,2*R*)-1-(2,5-Dihydroxy-2-oxo-2-λ-5-[1,3,2]dioxaphosphinan-5-yl)-butane-1,2,3,4-tetraol 11. Sodium borohydride (4.6 mg, 0.12 mmol) was added to a stirred solution of **9** (30 mg, 0.11 mmol) in D_2O (5 mL). The reaction was monitored by ^{31}P NMR. After 2 h the starting material was completely consumed. The reaction was quenched by the addition of Dowex® (H^+ form). The crude reaction mixture was freeze-dried after filtration to afford glucitol **11**. ^1H NMR (D_2O) δ 4.76–4.73 (2H, m), 4.40–4.25 (4H, m), 3.82–3.70 (1H, m), 3.41–3.34 (1H, m), 3.19–3.06 (1H, m); ^{13}C NMR (D_2O) δ 80.1, 75.8, 74.1, 72.8, 72.1, 69.3, 64.1; ^{31}P NMR (D_2O) δ −2.1; LRMS (ES) Calcd for $C_7H_{14}O_9P$ (M−1) 273.1 found 273.2; HRMS (ES) Calcd for $C_7H_{14}O_9P$ (M−H^-) 273.0319 found 273.0313.

4.1.26. (1*S*,2*R*)-1-(2,5-Dihydroxy-2-oxo-2-λ-5-[1,3,2]-dioxaphosphinan-5-yl)-butane-1,2,4-triol 12. Sodium borohydride (3.3 mg, 0.086 mmol) was added to a stirred solution of **10** (20 mg, 0.078 mmol) in D_2O (5 mL). The reaction was monitored by ^{31}P NMR. After 2 h the starting material was completely consumed. The reaction was quenched by the addition of Dowex® (H^+ form). The crude reaction mixture was freeze-dried after filtration to afford glucitol **12**. ^1H NMR (D_2O) δ 4.50–3.81 (4H, m), 3.21–3.02 (4H, m), 2.16–1.97 (2H, m); ^{13}C NMR (D_2O) δ 82.9, 80.2, 72.8, 72.6, 56.5, 50.2, 42.4; ^{31}P NMR (D_2O) δ −1.8 (br s); LRMS (ES) Calcd for $C_7H_{15}O_8P$ (M−1) 255.1 found 255.10; HRMS (ES) Calcd for $C_7H_{14}O_8P$ (M−H^-) 255.0265 found 255.0275.

4.2. In vitro assay of isolated human MIP synthase

4.2.1. Purification of recombinant human MIP synthase. Human MIP synthase was purified as previously described.[16] Briefly, *Escherichia coli* strain containing the recombinant pRSETA/*hINO1* construct was grown at 37 °C to an *A*550 of 0.4. Production of recombinant protein was induced by 1 mM isopropyl-1-thio-D-galactopyranoside for 3 h. Cells were lysed by sonication, and supernatant was

loaded to Ni^{2+} column (invitrogen). The column was then washed five times with buffer A (50 mM Na_2PO_4, 0.5 M NaCl, 20 mM imidazole, pH 7.0), and the protein was eluted with buffer B (50 mM Na_2PO_4, 0.5 M NaCl, 250 mM imidazole, pH 8.0).

4.2.2. MIP Synthase assay. Purified MIP synthase activity was determined by the rapid colorimetric method of Barnett et al.[21] with minor modification. Purified protein was suspended in the reaction buffer (100 mM Tris acetate, pH 8.0, 0.8 mM NAD, 2 mM dithiothreitol, 14 mM NH_4Cl) containing the inhibitor present in various concentrations (0, 50 nM, 5μM and 5 mM). After addition of 5 mM glucose 6-phosphate to a final volume of 150 μL, the reaction mixture was incubated for 1 h at 37 °C. The reaction was terminated by the addition of 50 μL of 20% (w/v) trichloroacetic acid and kept on ice for 10 min. The precipitated protein was removed by centrifugation. Supernatant (200 μL) was incubated with 200 μL of $NaIO_4$ for 1 h. Na_2SO_3 (200 μL of 1 M) was then added to the supernatant to remove the excess $NaIO_4$. For the measurement of phosphate, a 600-μL reagent mixture (240 μL of H_2O, 120 μL of 2.5% ammonium molybdate, 120 μL of 10% ascorbic acid, and 120 μL of 6 N sulfuric acid) was added and incubated for 1 h at 37 °C. The absorbance was measured at 820 nm, and activity was determined by the amount of inorganic phosphate liberated. For each assay, a second aliquot of the sample was measured for phosphates not released by $NaIO_4$ treatment to control for phosphatase activity. This value was subtracted from the experimental sample to obtain MIP synthase activity. Data represent the average of two independent experiments.

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