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Probing *myo*-inositol 1-phosphate synthase with multisubstrate adducts†

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The synthesis of a series of carbohydrate–nucleotide hybrids, designed to be multisubstrate adducts mimicking *myo*-inositol 1-phosphate synthase first oxidative transition state, is reported. Their ability to inhibit the synthase has been assessed and results have been rationalised computationally to estimate their likely binding mode.

Introduction

myo-Inositol 1-phosphate synthase (mIPS) catalyses the first step in inositol biosynthesis, and carries out the conversion of D-glucose 6-phosphate (G6P) into *myo*-inositol 1-phosphate (mIP).[1] The structural gene encoding mIP synthase (*INO1*) has been identified, isolated and cloned from several prokaryotic and eukaryotic microorganisms.[2,3] The gene sequence of *INO1* exhibits a high degree of homology amongst eukaryotes which drops when compared to prokaryotes.[2,4] Stoker *et al.* have demonstrated that a mutant *Mycobacterium tuberculosis* lacking *INO1* is only viable in the presence of extremely high levels of exogenous inositol.[5] The limited degree of homology and the innate need for mIPS activity in bacterial strains like *M. tuberculosis* renders mIPS an attractive therapeutic target for the identification of novel antiproliferative agents. Similarly, mIPS has been proposed as possible target for bipolar drugs.[6] As such, a carefully rationalised understanding of the eukaryotic mIPS' mechanism and its divergence from the prokaryotic mIPS, is crucial if one is to facilitate such endeavours. From a mechanistic perspective,[7,8] mIPS converts G6P into mIP (Scheme 1) in 4 steps within one catalytic cycle, using NAD as a prosthetic group. The mIPS/NAD complex catalyses the oxidation of the C5 hydroxyl of G6P presented in its opened form in the cofactor-bound enzyme catalytic pocket with the concomitant reduction of NAD to NADH. The subsequent enolisation, proposed to be substrate-assisted, precedes the intramolecular aldol condensation and the subsequent reduction of the resulting inosose-2-phosphate to mIP with the concomitant oxidation of NADH to NAD.[9]

While this catalytic sequence has been validated, the crystallographic analyses of eukaryotic mIPS with various inhibitors,[1,4,10] substrate analogues[11] and cofactors[12] have provided structural information which have raised more questions than anticipated. For instance, mIPS isolated from *Saccharomyces cerevisiae* was co-crystallised in the presence of NAD with 2-deoxyglucitol 6-phosphate (**1**, Fig. 1) and with 2-deoxy-D-glucitol 6-(*E*)-vinylhomophosphonate (**2**, Fig. 1), respectively and the mechanistic consequences rationalised.[1] Both G6P analogues are potent competitive inhibitors of mIPS; however their binding modes were very distinct. 2-Deoxy glucitol 6-phosphate (**1**) is a competitive inhibitor of mIPS with K_i in the mid-micromolar range against eukaryotic mIPS, binding the NAD-mIPS complex in almost a head-to-tail type binding (Type 1 binding, Fig. 2). 2-Deoxy-D-glucitol 6-(*E*)-vinylhomophosphonate, the most potent mIPS competitive inhibitor at submicromolar concentration is a slow-binding inhibitor and when compared to the binding mode of (**1**), (**2**) binds in what is anticipated to be a substrate-like binding mode (Type 2 binding, Fig. 2). Additionally and unlike (**1**), the glucitol (**2**) was shown to be oxidised by mIPS/NAD to yield a mIPS/NADH/enone phosphonate complex, mimic of the mIPS/NADH/5-ketoglucose 6-phosphate productive complex **A** (Scheme 1), further supporting the substrate-like binding mode of (**2**). These results, along with the crystallographic analyses strongly supported the proposal that 2-deoxy-D-glucitol 6-(*E*)-vinylhomophosphonate binding mode was best at mapping mIPS catalytic and binding sites, both for NAD and its natural substrate, glucose 6-phosphate. Yet, the limited and controversial nature of the chemical and structural data available on mIPS catalytic site, compounded by the major induced-fit movements occurring within mIPS upon the binding of NAD to the *apo*-mIPS

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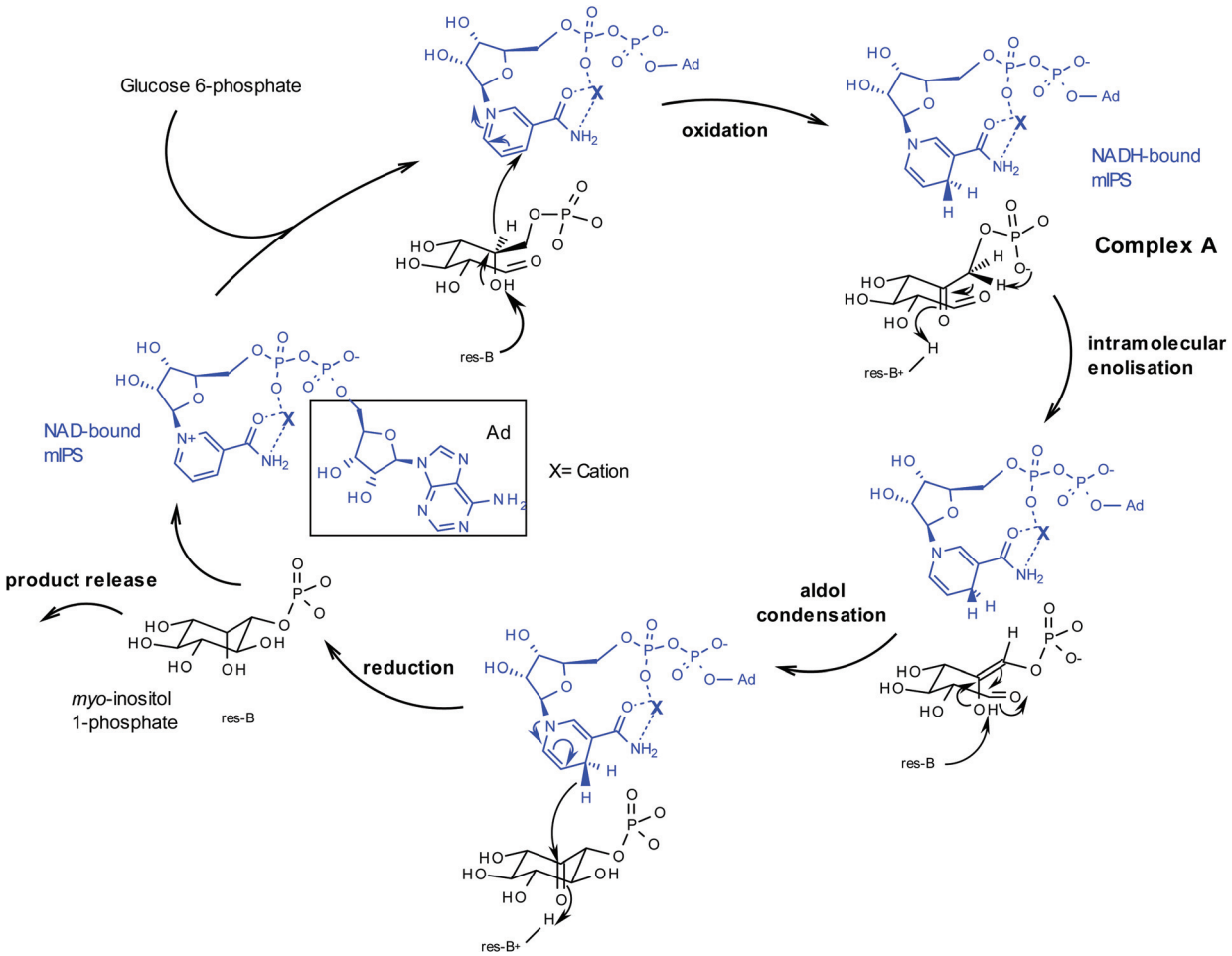


Scheme 1 mIPS catalytic cycle.

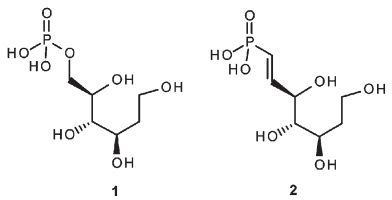


Fig. 1 2-Deoxy-D-glucitol 6-phosphate (**1**) and 2-deoxy-D-glucitol 6-(*E*)-vinyl-homo-phosphonate (**2**), low micromolar inhibitors of eukaryotic mIPS.

and the binding of G6P to the mIPS/NAD complex, implies that new approaches to designing high affinity mIPS ligands capable of providing structural information both at the NAD and the G6P binding sites would be highly valuable.

We decided to employ a multisubstrate adduct (MA) approach to the design of such potential ligands and use the transition state of the first oxidative step of the catalytic cycle as template. The design of the multisubstrate adducts was based on the transition state for which the C-5 hydrogen of G6P is transferred as hydride to NAD.[8,13] The selection of synthetic targets was then guided by chemical feasibility. To support the MA approach, it must be noted that mIPS/NAD

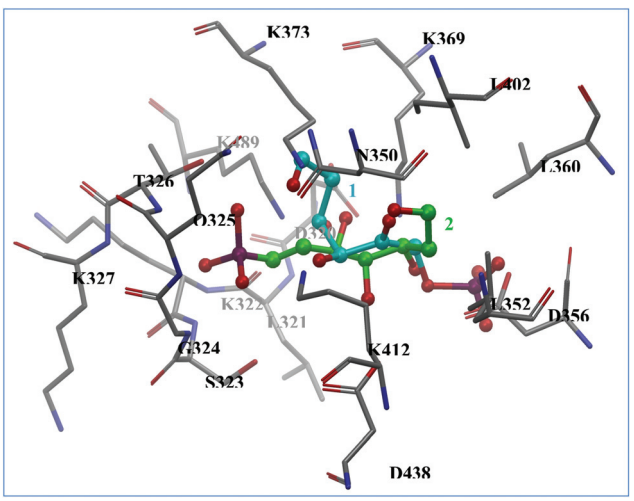


Fig. 2 Depiction of head-to-tail binding, through backbone superimposition of 1rm0 and 1jki mIPs structures.

exchange studies on a mixture of 5-^{2}H-G6P/G6P have shown that tightening of the complex occurs in the early stages of the catalysis, with no scrambling of the deuterium atom upon

turnover to L-2-^{2}H-mIP and L-mIP respectively (Fig. 1).[14] Therefore, we proposed to generate a class of multisubstrate adducts where the carbohydrate moiety of G6P was to be linked to an aromatic ring incorporated in a nucleosidic linkage with a riboside moiety. Such a molecule would possess the features of the phosphosugar substrate and of the nucleotide cofactor, both required for a multisubstrate type binding to mIPS. It is anticipated that upon mIPS binding, such MA would offer high levels of stabilisation through interactions with the individual substrate and cofactor binding pockets' residues, as well as high level of specificity. Ideally, an adduct of this type could incorporate the adenosine moiety of NAD^+ as the adenine nucleobase which has been shown to provoke a major conformational shift upon binding of NAD^+ through specific and selective interactions and as such being deeply buried into the NAD^+ binding pocket.[12] However, adenosine diphosphate ribose (ADPR) was found to be a poor inhibitor of mIPS, thus indicating the importance of the ribosyl nicotinamide moiety for tight binding and potency. Additionally, since it is thought that it is the open form of G6P, and not the cyclic anomer,[7] which binds NAD^+-bound mIPS, the carbohydrate moiety most likely to provide the best binding potential with mIPS as part of an adduct structure is therefore the reduced sugar form, *i.e.* glucitol. Finally, on all occasions when the 2-deoxy sugar series were synthesised and evaluated as mIPS's inhibitors, increased potency was achieved over the hydroxylated parent series. It is therefore reasonable to consider also a set of multisubstrate adducts where the 2-deoxy series can be assessed and compared for potency against the hydroxylated sugar parents.

The multisubstrate adduct was to also incorporate features from NAD, in particular that of the nicotinamide nucleotide part of the cofactor, which was to be linked by a triazole moiety to the glucitol moieties.[15,16] A methylenoxy was chosen to bridge the glycon units and to compensate for the spatial and rotational restrictions introduced by the triazolide moiety. As such, the selected triazolide-type linkage (Fig. 2) provided

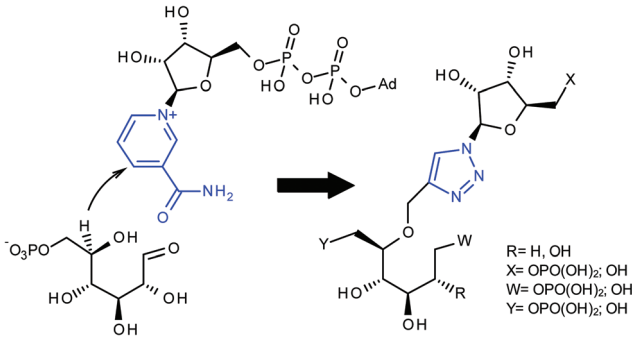


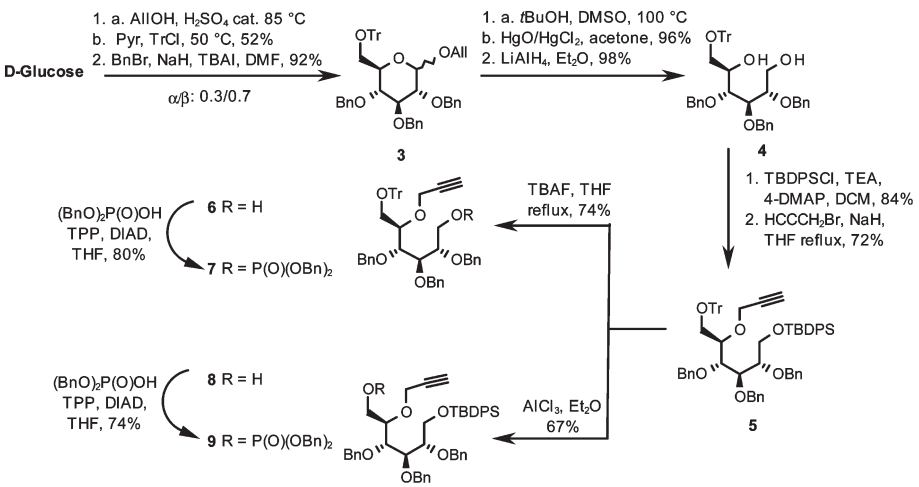
Fig. 3 Rationale for MAs design.

structural flexibility between the two multisubstrate adduct subunits while offering H-bond interactions and metal coordination opportunities.[1] Finally, since (**1**) and (**2**) adopt opposite binding conformations inside the mIPS active site, and that the initial binding conformation of the substrate remains unknown, the target MAs were designed to address such binding versatility. Therefore, the structure of the multisubstrate adducts was devised to include a glucitol or 2-deoxyglucitol moiety which incorporated a phosphoryl moiety on the primary alcohols of the modified glucitol, so to provide opportunities for the "head-to-tail binding" like for 2-deoxy glucitol 6-phosphate (**1**) or for the productive binding of 2-deoxy-D-glucitol 6-(*E*)-vinylhomophosphonate (**2**) with NAD-mIPS. Therefore, a phosphate monoester group was introduced at the positions X, Y and W (Fig. 3) to generate the series of mono- and di-phosphorylated MAs.

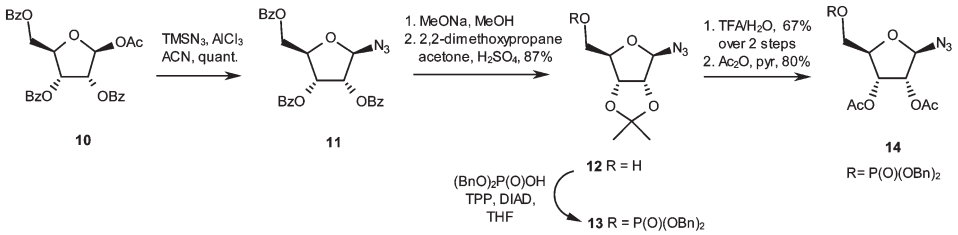
Results and discussion

Synthesis

In order to synthesise the glucitol based MA series, we first focused on accessing two types of precursors, the alkynes (**7**) and (**9**) (Scheme 2) and the furanosyl azides (**11**) and (**14**)



Scheme 2 Synthesis of glucitol precursors of MAs.



Scheme 3 Synthesis of furanosyl azide precursors of MAs.

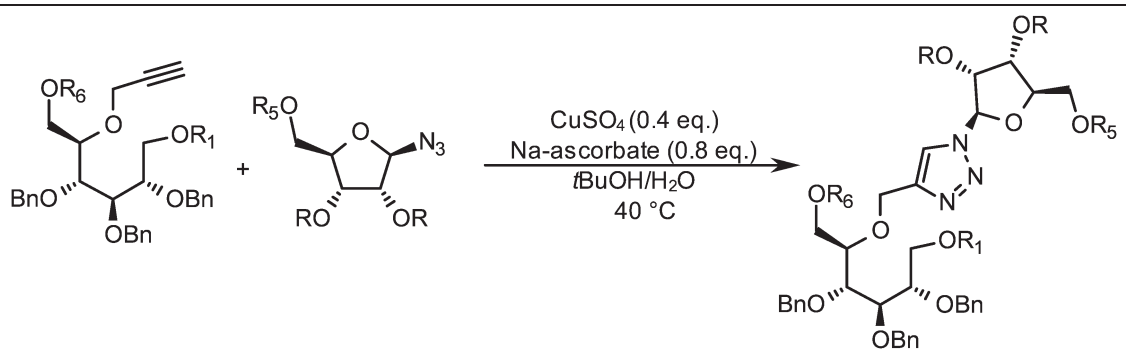
(Scheme 3). These two families of precursors were then coupled *via* copper(I)-catalysed triazolide chemistry leading to the fully-protected MA precursors, which were subsequently deprotected. The protecting group strategy adopted for this synthetic sequence aimed at accessing the pure MAs from the pure benzylated parents, so that a quantitative deprotection step by hydrogenolysis could be conducted.

The alkyne intermediate (**5**), precursor of (**7**) and (**9**), was obtained *via* a 7-step route, starting from an α/β D-glucose mixture. The protection of the anomeric hemiacetal with an allyl group under Fisher conditions was followed by the protection of the C6-position with trityl chloride affording allyl 6-*O*-trityl-D-glucopyranoside intermediate in 52% overall yield.[17] The remaining free hydroxyl groups were then benzylated giving allyl-2,3,4-tri-*O*-benzyl-6-*O*-trityl-D-glucopyranoside (**3**) in excellent 92% yield.[18] After the removal of the allyl group, the reduction of the pyranose ring yielded 2,3,4-tri-*O*-benzyl-6-*O*-trityl-D-glucytol (**4**) almost quantitatively. *tert*-Butyldiphenylsilyl chloride was used to selectively protect the primary alcohol in 84% yield and the propargylation of the secondary alcohol at the C5 position was achieved in 72% yield by using propargyl bromide and sodium hydride in THF at reflux. The propargyl intermediate (**5**) was then selectively deprotected at the C6 and C1 positions by treatment with $AlCl_3$ in diethyl ether to access the C6-deprotected propargylated glucitol (**6**), or by treatment of (**5**) with TBAF in THF to access the C1-deprotected parent (**8**), respectively. Both intermediates (**6**) and (**8**) were phosphorylated *via* a Mitsunobu-type reaction using the free acid of dibenzylphosphate diester, leading to the two glucitol precursors (**7**) and (**9**) in good 80% and 74% yields, respectively. Other phosphorylation methods including phosphochloridite and phosphoramidite P(III) chemistry and phosphochloridate and pyrophosphate P(V) chemistry proved either unsuccessful or extremely low yielding.

Two furanosyl azide units were synthesized with the aim to incorporate a phosphate moiety at the C5 position of the furanosyl moiety. Thus, by treatment of (**10**) with azido-trimethylsilane with $AlCl_3$ in diethyl ether, the first furanosyl azide unit (**11**) was obtained quantitatively.[16] After removal of the benzoate groups, the secondary hydroxyl groups were protected by using 2,2-dimethoxypropane in acetone, leading to (**12**) in 87% yield over two steps. The phosphate moiety was then introduced using the Mitsunobu protocol, and the isopropylidene moiety removed in the presence of TFA to facilitate purification and affording the diol intermediate in 67% yield over 2 steps. The furanosyl hydroxyl groups had to be acetylated to form (**14**), as its solubility in organic solvents had a dramatic effect on the reaction rates during the triazolide formation by click chemistry.

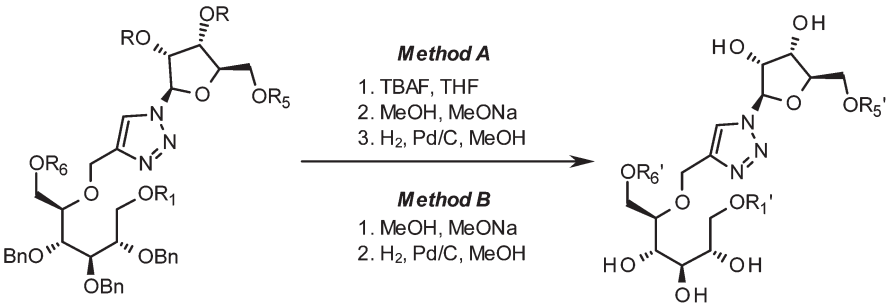
The cyclisation between the different precursors (**5**), (**7**), (**9**) and (**11**), (**14**) occurred upon treatment with a catalytic amount of copper sulfate and sodium ascorbate in a *t*BuOH–H_2O

Table 1 Synthesis of fully-protected MAs **(15)–(19)** by click chemistry



Alkyne	R_6	R_1	Azide	R_5	R	Product	Yield
(**5**)	Tr	TBDPS	(**11**)	Bz	Bz	(**15**)	(62%)
(**5**)	Tr	TBDPS	(**14**)	$P(O)(OBn)_2$	H	(**16**)	(37%)
(**7**)	Tr	$P(O)(OBn)_2$	(**14**)	$P(O)(OBn)_2$	Ac	(**17**)	(73%)
(**9**)	$P(O)(OBn)_2$	TBDPS	(**11**)	Bz	Bz	(**18**)	(58%)
(**9**)	$P(O)(OBn)_2$	TBDPS	(**14**)	$P(O)(OBn)_2$	Ac	(**19**)	(62%)

Table 2 Synthesis of MAs **(21)–(24)** from the fully protected MA precursors **(16)–(19)**



	Substrate				Product			
	R_6	R_5	R	R_1	R_6'	R_5'	R_1'	(%)[a]
16	Tr	$P(O)(OBn)_2$	Ac	TBDPS	H	$P(O)(OH)_2$	H	**23**[b] (84%)
17	Tr	$P(O)(OBn)_2$	Ac	$P(O)(OBn)_2$	H	$P(O)(OH)_2$	$P(O)(OH)_2$	**24**[c] (94%)
18	$P(O)(OBn)_2$	Bz	Bz	TBDPS	$P(O)(OH)_2$	H	H	**21**[b] (81%)
19	$P(O)(OBn)_2$	$P(O)(OBn)_2$	Ac	TBDPS	$P(O)(OH)_2$	$P(O)(OH)_2$	H	**22**[b] (73%)

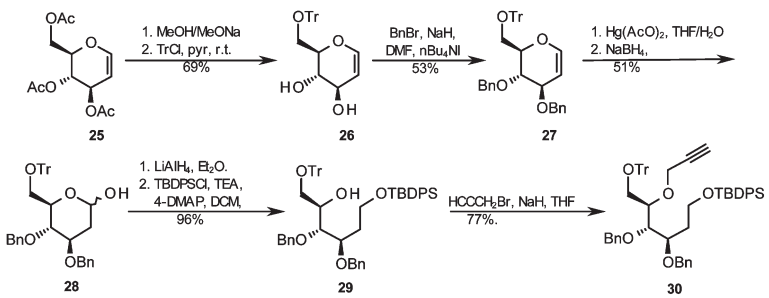
[a] Overall isolated yield. [b] Deprotection with Method A. [c] Deprotection with Method B.

mixture, heated at 40 °C for a period of 6–8 hours (Table 1) in low (*e.g.* (**16**) in 37%) to good yields (*e.g.* (**17**) in 73%). In order to force this reaction, the furanosyl azide was used in slight excess.

It is anticipated that the lower yields observed for the formation of (**16**) are to be associated with the likely hydrophilic solvation level of the partially protected highly polar phosphosugar, thus limiting its interactions with the more lipophilic glucitol propargyl reagents. Similar results were obtained for the deoxy-glucitol series, *e.g.* compound (**35**).

The deprotection sequence proved to be crucial to the successful completion of this class of MAs' synthesis. Initially, the following sequence was selected; ester deprotection, followed by trityl or TBDPS deprotection followed by hydrogenolysis. However, this strategy required workup and purification by column chromatography at each step, resulting in noticeable mass losses. Crucially, it was noticed that compounds bearing a TBDPS group and those bearing a trityl moiety did not generate the same type of sugar-based impurities, each class leading to a different type of disproportionation, in particular with regards to phosphate moiety losses. Therefore, the specific sequence was developed for the TBDPS-protected MAs (Table 2, Method A). For compounds (**15**), (**18**) and (**19**), the acyl moieties were first removed with sodium methoxide in methanol, and the TBDPS was then removed using TBAF in THF. Water was added prior to the removal of THF to prevent product decomposition and allow for the extraction of the respective products in DCM. The untimely removal of THF under reduced pressure and increased ionic strength caused the loss of the phosphate tri-ester moiety at the C6 position. After purification by chromatography column, the partially protected phosphorylated intermediates underwent hydrogenolysis yielding (**21**) and (**22**) in good (73–94%) overall yields.

The trityl protected MAs (**16**) and (**17**) were deprotected *via* a two-step process consisting in the use of sodium methoxide in methanol followed by hydrogenolysis (Table 2, Method B). While the trityl moiety could be deprotected under mild acidic conditions in (**16**),[19] these conditions also yielded the removal of the phosphate tri-ester group at the C1 position in the protected MA (**17**). This phosphate hydrolysis was assumed to occur *via* an intramolecular nucleophilic substitution initiated by the initial release of C6–OH group and acid catalysed activation of the P=O bond.[20] The trityl group removal was therefore carried out concurrently to the removal of the benzyl



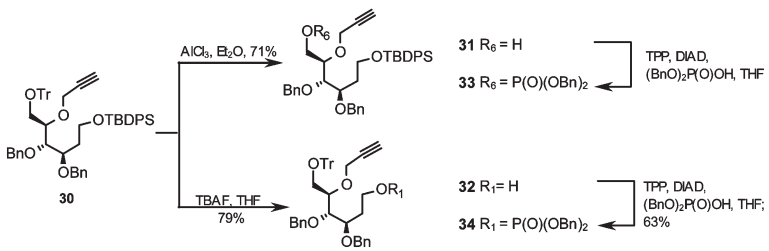
Scheme 4 Synthesis of 2-deoxy glucitol precursors of MAs.

groups of the phosphoesters at the hydrogenolysis step leading to (**24**) in excellent 94% overall yield. The removal of the first benzyl moiety of the phosphate group is fast, thus generated a more stable phosphodiester intermediate. The *in situ* generation of the phosphoric acid diester is then sufficient to initiate the acid-catalysed removal of the trityl group, revealing the C6–OH moiety which remains unreactive towards the partially and fully deprotected phosphate ester moieties.

Triacetylated D-glucal (**25**) was used as starting material for the preparation of the 2-deoxy series of MAs, as 2-deoxy-D-glucose was not suitable as starting material for its implementation in the strategy developed for the D-glucose series (Scheme 4). As such, the partially protected glycal (**26**) was obtained following removal of the acetate groups by treatment with sodium methoxide in methanol and selective protection of the primary hydroxyl with trityl chloride. Following the benzylation of (**26**) in 53% yield, the fully protected glycal (**27**) was subjected to a mercuration–reductive demercuration sequence in order to convert the D-glucal derivative into its 2-deoxy-D-glucose analogue (**28**). This was achieved in 51% yield. From this stage onwards, the same synthetic sequence as that developed for the D-glucose series was implemented.

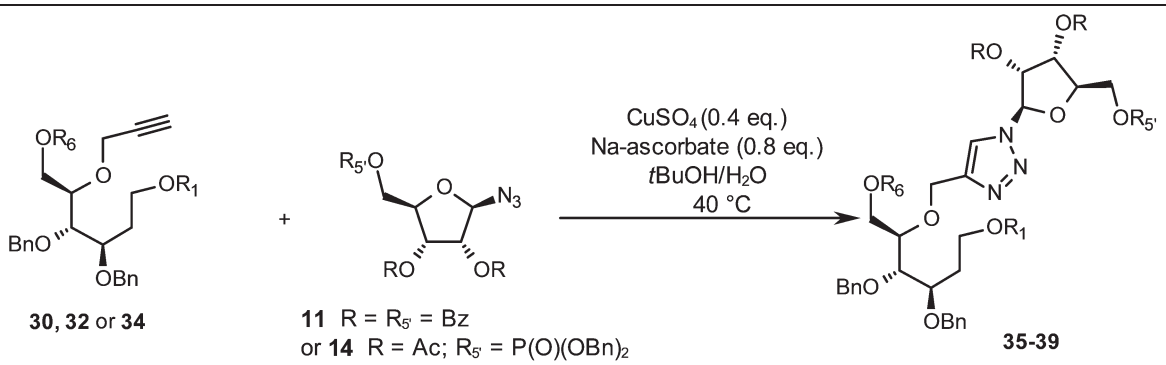
The 2-deoxy progargylated glucitol (**30**) was selectively deprotected at the C6 and C1 positions by treatment with aluminium trichloride in diethyl ether and TBAF in THF, to yield the propargylated glucitols (**31**) and (**32**), respectively (Scheme 5). Both intermediates (**31**) and (**32**) were phosphorylated *via* a Mitsunobu-type reaction using dibenzylphosphate leading to the two glucitol precursors (**33**) and (**34**), respectively. At this stage, problems were encountered during purification, in particular for the compound (**33**). Both the by-product DIHD and the C6-phosphorylated alkyne (**33**) co-eluted regardless of the eluent system used. Therefore, isolation of compound (**33**) as a pure product was not possible under standard silica chromatographic methods. Nonetheless, the diisopropyl hydrazodicarboxylate (DIHD) contaminated material could be used in the subsequent steps.

The coupling between the different types of propargylated precursors (**30**), (**33**) and (**34**) and the azido-furanosides previously synthesised occurred by using catalytic amount of copper sulfate and sodium ascorbate in a *t*BuOH–H_2O mixture at 40 °C for 6–8 hours (Table 3) in presence of a slight excess of the azido reagent. While facile isolation of the cyclised products was achieved for most reactions, it was necessary to first



Scheme 5 Selective deprotection and phosphorylation of the precursors to the 2-deoxy glucitol MAs series.

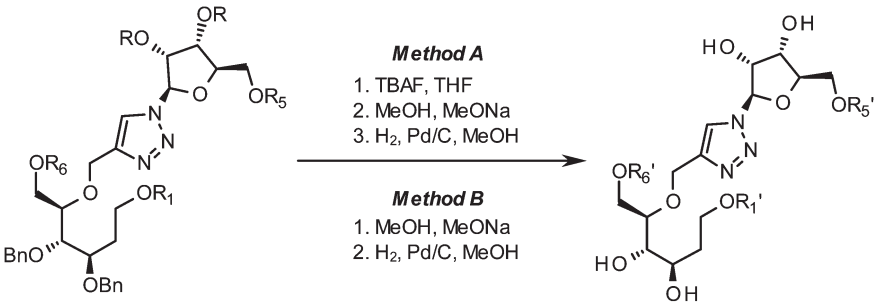
Table 3 Synthesis of fully-protected 2-deoxy glucitol MAs **(35)–(39)** by click chemistry



Alkyne	R_6	R_1	Azide	Product	R	$R_{5'}$
30	Tr	TBDPS	**14**	**35**[a] (28%)	H	$P(O)(OBn)_2$
33	$P(O)(OBn)_2$	TBDPS	**14**	**36** (39%)	Ac	$P(O)(OBn)_2$
33	$P(O)(OBn)_2$	TBDPS	**11**	**37** (71%)	Bz	Bz
34	Tr	$P(O)(OBn)_2$	**14**	**38** (62%)	Ac	$P(O)(OBn)_2$
34	Tr	$P(O)(OBn)_2$	**11**	**39** (44%)	Bz	Bz

[a] To facilitate subsequent purification, the acetylated triazolide product obtained from (**30**) and (**14**) had to be deprotected following work up using MeOH/MeONa.

Table 4 Synthesis of MAs **(40)–(44)** from the fully protected MA precursors **(35)–(30)**



	Substrate				Product			
	R_6	R_5	R	R_1	R_6'	R_5'	R_1'	(%)[a]
35	Tr	$P(O)(OBn)_2$	H	TBDPS	H	$P(O)(OH)_2$	H	**42**[b] (75%)
36	$P(O)(OBn)_2$	$P(O)(OBn)_2$	Ac	TBDPS	$P(O)(OH)_2$	$P(O)(OH)_2$	H	**43**[b] (76%)
37	$P(O)(OBn)_2$	Bz	Bz	TBDPS	$P(O)(OH)_2$	H	H	**44**[b] (68%)
38	Tr	$P(O)(OBn)_2$	Ac	$P(O)(OBn)_2$	H	$P(O)(OH)_2$	$P(O)(OH)_2$	**40**[c] (90%)
39	Tr	Bz	Bz	$P(O)(OBn)_2$	H	H	$P(O)(OH)_2$	**41**[c] (87%)

[a] Overall isolated yield. [b] Deprotection with Method A. [c] Deprotection with Method B.

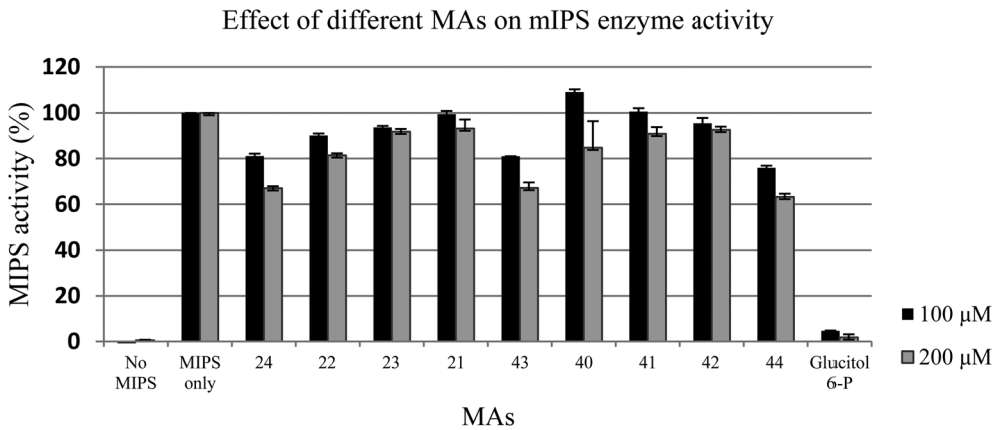


Fig. 4 mIPS inhibition by MAs assayed at 100 and 200 micromolar concentrations.

remove the acetate groups to isolate the corresponding protected MA for the 2-deoxy glucitol adduct (**35**).

The same sequence of deprotection as that developed for the glucitol series was applied to the tritylated and silylated protected 2-deoxyglucitol MAs to yield the fully deprotected 2-deoxy parent series, compounds **(40)–(44)**. Similar yields to those obtained for the glucitol MAs series were obtained for the preparation of the 2-deoxy MAs (Table 4).

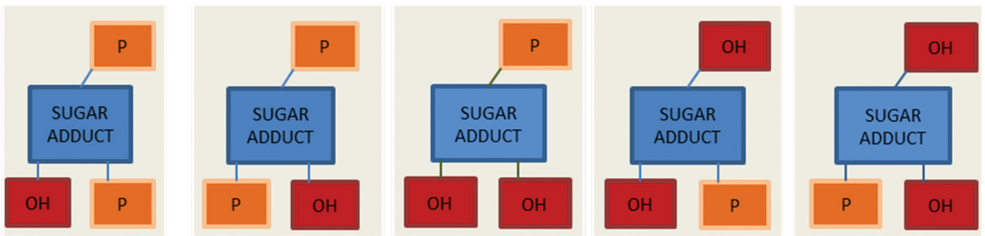
Biological evaluation

All MAs were evaluated against purified yeast recombinant mIPS enzyme as inhibitors of G6P conversion to mIP. Purified protein was suspended in the reaction buffer and incubated in the presence of the MAs in various concentrations (0, 100 μM and 200 μM). Glucose 6-phosphate (5 mM) was then added and the reaction mixture was incubated for 1 h at 37 °C. The remaining mIP synthase activity was determined by the rapid colorimetric method of Barnett *et al.*[22] with minor modifications.

All MA compounds thus synthesised and assayed against mIPS displayed some level of inhibition, even though modest, at high micromolar levels in a concentration-dependent manner (Fig. 4).

Table 5 provides a summary of the inhibitory properties of the series of MAs thus far synthesised, with the remaining activity being measured at two concentrations (100 and 200 μM) of MAs in the enzymatic assay solution. As indicated in Table 5, compounds (**40**) and (**41**), both 2-deoxy glucitol based MAs, lack inhibitory properties. This would indicate that in the case of the deoxy series, the phosphate positioned

Table 5 Inhibitory properties of the schematically presented phosphorylated MAs

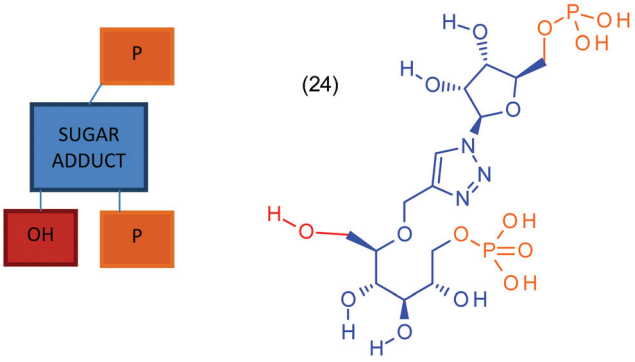


Series	Inhibitor concentration	MAs	%[a]	MAs	%[a]	MAs	%[a]	MAs	%[a]	MAs	%[a]
Glucitol series	100 μM	**24**	81	**22**	90	**23**	93			**21**	99
	200 μM		67		81		92				93
2-Deoxy-glucitol series	100 μM	**40**	109	**43**	81	**42**	95	**41**	101	**44**	76
	200 μM		84		67		93		90		63

[a] % mIPS activity remaining upon incubation.

next to the 2-deoxy carbon has a detrimental effect on recognition by mIPS. However, a phosphate group located at the C6 position of the 2-deoxyglucitol end of the MAs restores inhibition, whether or not the riboside end is phosphorylated, as demonstrated by the inhibitory properties displayed by (**43**) and (**44**). For comparison, 2-deoxy glucitol 6-phosphate was also incubated under the same conditions, and less than 5% mIPS activity was observed at these concentrations, consistent with the fact that it is a potent inhibitor of mIPS.

Graphical representation using (**24**) as example:



Importantly, the introduction of the riboside adduct at the C5-position of the glucitol 6-phosphate moiety has a detrimental impact on the overall inhibitory activity of this class of compounds. This might be due to the removal of the C5-hydroxyl-mIPS hydrogen bonding observed between 2-deoxy glucitol 6-phosphate and mIPS, reducing the overall mIPS binding affinity for the MAs. Alternatively, the flexibility of the MAs might be a major limiting factor with regards to the initial enzyme–inhibitor recognition. It must also be noted that NAD is thought to bind the *apo*-enzyme, which then becomes conformationally "ready" for G6P binding. In a standard assay, the multisubstrate adducts might not be able to compete effectively with NAD, which possesses a K_m in the micromolar range. In an attempt to address some of these aspects of MAs' binding to mIPS, we conducted pre-incubation experiments, whereby *apo*-mIPS and the MAs were pre-incubated for 15 min prior to the addition of NAD, the cofactor and G6P, the substrate. No difference in the overall remaining enzyme activity was observed, indicative of a truly reversible equilibrium and the absence of long-lived enzyme inhibitor complex (*i.e.* low K_{off}). This also indicated that even if the flexibility of the MAs was detrimental to recognition by mIPS, it was not a limiting factor with regards to mIPS inhibition. When the 5-phosphate riboside is the only anionic moiety in the MAs (*i.e.* (**23**) and (**42**)), no concentration-dependent inhibition is observed, indicative that the remaining sugar moiety has no impact on mIPS binding. On the other hand, when there is no phosphate on the furanoside moiety, then the hexose series display a consistent inhibitory trend. This would indicate that the recognition process is glucitol phosphate-driven rather than riboside phosphate-driven.

We conducted some docking experiments using 1rm0 and 1jki crystallographic data,[1,11] for which mIPS was either co-crystallised in the presence of 2-deoxy-D-glucitol 6-(*E*)-vinylhomophosphonate or 2-deoxy glucitol 6-phosphate, respectively. On all accounts, the docking scores were better when 1rm0 was used. Importantly, with 1jki, the glucitol end of all the MAs, except (**41**), appears to dock preferentially in the NAD binding pocket, with the riboside end being accommodated in the glucose 6-phosphate binding pocket. With 1rm0, all MAs tested show binding of the riboside moiety in the NAD pocket for nicotinamide riboside with the (2-deoxy)-glucitol moiety fitting in the substrate binding site. To illustrate this, the docked poses of (**43**) in 1rm0 and 1jki are found in Fig. 5.

Importantly, the same residues involved in the interaction between the two known potent inhibitors of mIPS remain apparently involved in the preferred coordination of the MAs when high docking scores are observed. Additionally, the

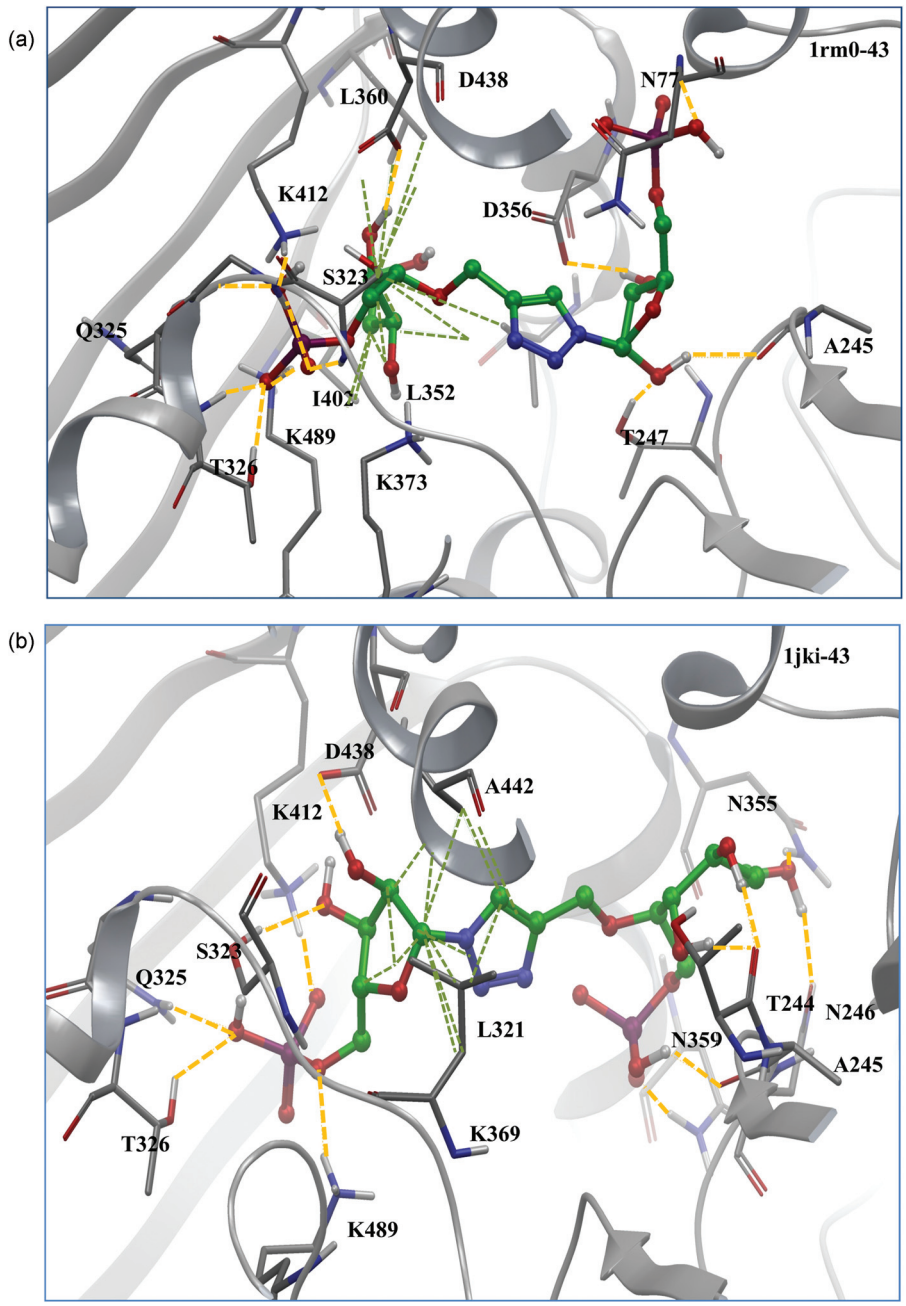


Fig. 5 Interaction of compound (**43**) with mIPS, using the conformation adopted in the crystal structure (a) 1rm0 and (b) 1jki.

compounds which appear to dock best in the anticipated binding mode (*i.e.* occupancy of both the nicotinamide riboside binding pocket and the glucose 6-phosphate binding pocket by the corresponding MAs moieties), are also the compounds which display the best inhibition against mIPS, thus supporting the overall observation that 1rm0 provides a more informative and reliable crystallographic starting point of the mIPS binding pocket. The poses also indicate that to occupy both pockets, the MAs ether linker adopts a highly strained conformation which pushes the triazolide ring out of the hydrophobic pocket which surrounds the nicotinamide ring in NAD, adopting an almost perpendicular conformation. This compounded by the high MAs flexibility is likely to disfavour strong binding affinity with the mIPS binding pocket, and might be a leading factor for the overall poor potency amongst the MA inhibitors.

Conclusion

We have synthesised a series of carbohydrate–nucleotide hybrids, designed to be multisubstrate adducts, mimicking *myo*-inositol 1-phosphate synthase first oxidative transition state. We have shown that these compounds inhibited the

synthase at high micromolar levels, on par with the K_m of glucose 6-phosphate ($K_m \sim 1$ mM) and have used known crystallographic information to rationalize computationally their likely binding mode. It is possible that the stereochemistry at the C5-position of the hexitol derivatives, imposed by the use of a D-sugar as starting material, has had a detrimental effect of the overall stability of the enzyme–inhibitor complex, due to the adoption of an unfavourable conformation while fitting the carbohydrate/nucleotide binding pockets. The route to the C5-epimers of (**24**), (**43**) and (**44**), from (**4**) and (**29**) are now under investigation.

Experimental

All reactions requiring anhydrous or inert conditions were carried out under a positive atmosphere of argon in oven-dried glassware. Solutions or liquids were introduced in the round bottom flask using oven-dried syringes or cannula through rubber septa. All reactions were stirred magnetically using Teflon-coated stir bars unless otherwise specified. In the case requiring heating, the reactions were warmed using an electrically-heated silicon oil bath, and the stated temperature is the temperature of the bath. In the cases requiring −78 °C cooling, the reactions were chilled with a dry ice/acetone bath. Organic solutions obtained after an aqueous work-up and washed with brine (saturated solution of NaCl) were dried over $MgSO_4$. Removal of solvents was accomplished using a rotary evaporator at water aspirator pressure or under high vacuum (0.5 mmHg). Tetrahydrofuran and diethyl ether were distilled under argon from sodium benzophenone. Dichloromethane and pyridine were distilled over calcium hydride. *N*,*N*-Dimethylformamide (DMF) was distilled over barium oxide under reduced pressure or purchased from Sigma-Aldrich. All the solvents were stored under argon over activated Linde 4 Å molecular sieves. All other solvents and reagents were used as received from commercial suppliers or purified according to: Perrin, D.D.; Armarego, W.L.F.; Perrin, D.R. *Purification of Laboratory Chemicals*; Pargamon Press; New York, 1980. Chemicals were purchased from Sigma-Aldrich Chemical Company, Lancaster, Alfa Aesar, Maybridge or ACROS. Solvents for extractions and chromatography were of technical grade. Solvents used in reactions were freshly distilled from appropriated drying agents before use. All other reagents were recrystallised or distilled as necessary. Flash chromatography was carried out using Merck Silica (40–60 μ) and acid washed sand. ^{1}H, ^{13}C (decoupled) and 2D (H-COSY: 512 × 512, HMQC: 128 × 2048) NMR spectra were all recorded on Brüker avance DPX 500 and ^{31}P spectra were recorded on Brüker avance DPX 300. TMS (0 ppm, ^{1}H NMR), $CDCl_3$ (77 ppm, ^{13}C NMR) and HMPA (26.7 ppm, ^{31}P NMR) were used as internal references. High-resolution mass spectrometry (HRMS) was recorded on a VG Quattro Triple Quadropole Mass Spectrometer (ES). Optical rotations were measured on a Perkin-Elmer 341 polarimeter. IR data were recorded on a Perkin Elmer 1720X IR Fourier transform spectrometer.

The synthetic procedures to access compounds (**3**), (**4**), (**11**), (**25**), (**26**), (**27**), (**28**), and (**29**) and their respective characterisation have been reported in the ESI.†

General procedures

Procedure A: phosphorylation with dibenzylphosphate. To a solution of TPP (3.0 equiv.) in dry THF ($C \sim 1.0$ M) at 0 °C, DIAD (3.0 equiv.) was added and the mixture was stirred until a yellow solid had precipitated. Dibenzylphosphate (1.5 equiv.) was then added to the mixture and after being stirred at 0 °C for 5 minutes, a solution of substrate in dry THF ($C \sim 0.01$ M) was slowly added. The reaction mixture was stirred and allowed to warm to rt over a period of 12 hours. The THF was then removed under reduced pressure and the residue was dissolved in Et_2O ($C \sim 1$ mg mL^{-1}). The organic solution was washed with a saturated $NaHCO_3$ solution (3 × 1/3 VEt_2O mL), water (3 × 1/3 VEt_2O mL), brine (1/3 VEt_2O mL) and dried over $MgSO_4$. The filtrate was kept in a sealed flask at rt overnight. The white precipitate was filtered off and purified by flash column chromatography.

Procedure B: triazole formation by click chemistry. To a flask containing a suspension of alkyne (1.0 equiv.), the appropriate azide (1.1 equiv.) and a water–*t*BuOH mixture (1 : 1 v/v, $C \sim 0.05$ mmol mL^{-1}) was added a pre-mixed copper sulfate (0.2 equiv.) and sodium ascorbate (0.8 equiv.) aqueous solution (in a minimum of water). The resulting yellow reaction mixture was heated at 40 °C until the media turned light blue (6–12 hours). After being cooled, the reaction mixture was diluted with water (V = V water–*t*BuOH mL) and the aqueous suspension was extracted with EtOAc (3 × 1/3 V mL). The organic layer was then washed with water (3 × V mL) and then brine (V mL) and dried over $MgSO_4$. After removal of EtOAc under reduced pressure, the crude product was purified by silica column chromatography.

Procedure C (method B in Tables 2 and 4): deprotection of fully protected multisubstrates incorporating a Tr moiety. To a flask containing the appropriated fully protected and phosphorylated multisubstrate (1.0 equiv.) in MeOH ($C \sim 0.01$ M) was added MeONa (0.1 equiv., 25% wt in MeOH) at rt. The reaction mixture was stirred until the TLC showed that reaction reached completion (0.5–2 hours). MeOH was then removed under reduced pressure and the residue was dissolved in EtOAc (V = 2 × V MeOH mL). The organic solution was washed with water until pH 7. EtOAc was then removed under reduced pressure and the crude residue was dissolved in MeOH ($C' \sim 0.01$ M). To the organic solution was added Pd/C (10% of mass of starting material) and the reaction was stirred under a H_2 atmosphere (balloon) overnight. MeOH was then removed under reduced pressure and the residue was dissolved in water (V′ = V′ MeOH mL) and the aqueous layer was washed with EtOAc (3 × 1/2 V′ mL). The resulting aqueous solution was then freeze-dried to give the corresponding deprotected multisubstrate as a pure compound.

Procedure D (method A in Tables 2 and 4): deprotection of fully protected multisubstrates incorporating a TBDPS moiety. To a flask containing the appropriated fully protected and

phosphorylated multisubstrate (1.0 equiv.) in THF ($C \sim 0.01$ M) was added a solution of TBAF (1.1 equiv., 1 M in THF) dropwise. The reaction was monitored by TLC until it was shown to have reached completion. The reaction mixture was diluted with water ($V = 2 \times V_{THF}$ mL) and extracted with EtOAc (3 × 1/2 V mL). The organic layer was concentrated under reduced pressure and the residue was carefully purified by flash column chromatography ($CHCl_3$/EtOH: 1/0–8/2 v/v). The pure fractions containing products were concentrated and then dissolved in MeOH ($C \sim 0.01$ M) and a solution of MeONa in MeOH (0.1 equiv., 25% wt in MeOH) was added dropwise. The reaction was stirred until completion (1–2 hours). MeOH was then removed under reduced pressure and the residue was dissolved in EtOAc ($V = 2 \times V$ MeOH mL). The organic solution was washed with water until pH 7. EtOAc was removed under reduced pressure and the crude material was dissolved in MeOH ($C' \sim 0.01$ M). To the organic solution was added Pd/C (10% of mass of starting material) and the reaction was stirred under a H_2 atmosphere (balloon) overnight. The MeOH was then removed under reduced pressure and the residue was dissolved in water ($V' = V'_{MeOH}$ mL). The aqueous solution was washed with EtOAc (3 × 1/2 V′ mL), and was then freeze-dried to give the corresponding unprotected multisubstrate as a pure ^1H and ^{13}C NMR compound.

2,3,4-Tri-O-*benzyl-1*-O-tert-*butyldiphenylsilyl-5*-O-*propagyl-6*-O-*trityl-D-glucitol (**5**).* To a solution of (**3**) (8.00 g, 11.5 mmol) and 4-DMAP (0.28 g, 2.3 mmol) in dry DCM (100 mL) was added dry TEA (4.79 mL, 34.5 mmol). After stirring for 20 minutes, TBDPSCl (3.3 mL, 12.7 mmol) was syringed to the reaction mixture. The reaction was stirred overnight and then concentrated under reduced pressure. The residue was directly purified by silica column chromatography (Hex/EtOAc/TEA: 9/0.9/0.1 v/v/v) to give 2,3,4-tri-*O*-benzyl-1-*O*-*tert*-butyldiphenylsilyl-6-*O*-trityl-D-glucitol intermediate (9.00 g, 84%) as a white foam. ^1H (500 MHz, $CDCl_3$) δ 7.62–7.61 (4H, m, H_{Ar}), 7.45–7.42 (6H, m, H_{Ar}), 7.41–7.36 (2H, m, H_{Ar}), 7.32–7.12 (26H, m, H_{Ar}), 6.94–6.92 (2H, m, H_{Ar}), 4.63 (1H, d, J = 11.4 Hz, CH_2–Ph), 4.59 (1H, d, J = 11.4 Hz, CH_2–Ph), 4.54 (1H, d, J = 11.4 Hz, CH_2–Ph), 4.51 (1H, d, J = 11.4 Hz, CH_2–Ph), 4.32 (2H, s, CH_2–Ph), 3.99–3.95 (1H, m, H5), 3.89–3.76 (5H, m, H1, H2, H3, H4), 3.34 (1H, dd, J = 3.6 Hz, 9.5 Hz, H6), 3.25 (1H, dd, J = 5.7 Hz, 9.5 Hz, H6), 3.01 (1H, d, J = 4.8 Hz, OH), 1.03 (9H, s, 3 × CH_3 TBDPS). ^{13}C (125 MHz, $CDCl_3$) δ 143.9(3) (C_{Ar} Tr), 138.4, 138.0, 137.9 (C_{Ar} Bn), 135.6, 133.4 (CH_{Ar} TBDPS), 133.3(2) (C_{Ar} TBDPS), 129.6(2) (CH_{Ar} TBDPS), 128.7, 128.3, 128.2(2), 128.1, 127.9, 127.8, 127.6, 127.4(2), 127.0 (CH_{Ar}), 86.5 (C2), 79.6 (C5), 78.0 (C4), 74.0 (C3), 73.1, 72.9, 71.1 (CH_2–Ph), 64.8 (C1), 63.4 (C6), 26.9 (CH_3), 19.1 ($C(CH_3)_3$). HRMS ES$^+$-TOF: Calculated for $C_{62}H_{64}O_6SiNa$ $[M + Na]^+$ = 955.4370, found $[M + Na]^+$ = 955.4371. FTIR (cm^{-1}): 3434 (νO–H), 3290 (νC–H alkyne), 3031 (νC–HAr), 2875 (νC–H alkyl), 1071 (νC–O), 736 ($\delta Csp^3$3–H).

To a solution of 2,3,4-tri-*O*-benzyl-1-*O*-*tert*-butyldiphenylsilyl-6-*O*-trityl-D-glucitol (0.65 g, 0.70 mmol) in THF (20 mL) was added sodium hydride (0.028 g, 1.9 mmol, 60% in oil) portionwise. After 30 minutes at reflux, propargyl bromide (0.1 mL, 80% in toluene, 0.77 mmol) was added to the reaction. After a further 8 hours at reflux, the reaction was carefully quenched with water (10 mL) and concentrated under reduced pressure. The residue was then dissolved with EtOAc (10 mL) and the organic phase was isolated and washed with water (3 × 5 mL), brine (5 mL) and dried with $MgSO_4$. The dried extract was concentrated under reduced pressure. The crude product was purified by silica column chromatography (Hex/EtOAc/TEA: 9/0.9/0.1–8/1.9/0.1 v/v/v) to give **3** (0.50 g, 72%) as a colourless oil. ^1H (500 MHz $CDCl_3$) δ 7.66, 7.62 (4H, m, H_{Ar}), 7.47–7.44 (6H, m, H_{Ar}), 7.42–7.36 (2H, m, H_{Ar}), 7.34–7.15 (24H, m, H_{Ar}), 7.13–7.00 (2H, m, H_{Ar}), 6.89–6.87 (2H, m, H_{Ar}), 4.71–4.57 (4H, m, CH_2–Ph), 4.56–4.40 (2H, m, CH_2–Ph), 4.29–4.25 (1H, dd, J = 2.4 Hz, 15.5 Hz, CH_2–alkyne), 4.08 (1H, dd, J = 2.4 Hz, 15.5 Hz, CH_2–alkyne), 3.94–3.79 (6H, m, H2, H3, H4, H5, H1), 3.49–3.48 (1H, dd, J = 2.4 Hz, 10.4 Hz, H6), 3.31–3.27 (1H, dd, J = 5.6 Hz, 10.5 Hz, H6), 2.26 (1H, t, J = 2.4 Hz, CH≡C), 1.01 (9H, s, $C(CH_3)_3$). ^{13}C (125 MHz $CDCl_3$) δ 143.9(3) (C_{Ar} Tr), 138.8, 138.7, 138.4 (C_{Ar} Bn), 135.7, 135.6 (CH_{Ar} TBDPS), 133.6, 133.5 (C_{Ar} TBDPS), 129.6(2) (CH_{Ar} TBDPS), 128.8, 128.2, 128.1, 127.9, 127.8, 127.6(2), 127.3(2), 127.1, 126.9 (CH_{Ar}), 86.8 ($C(Ph)_3$), 80.6 (C2), 80.3 (C≡CH), 78.9 (C5), 78.4 (C3), 78.1 (C4), 74.4 (CH_2–Ph), 74.3 (CH≡C), 73.7 (CH_2–Ph), 73.2 (CH_2–Ph), 64.2 (C1), 63.2 (C6), 57.7 (CH_2–alkyne), 26.9 (CH_3), 19.2 ($C(CH_3)_3$). HRMS ES$^+$-TOF: Calculated for $C_{65}H_{66}O_6SiNa$ $[M + Na]^+$ = 993.4526, found $[M + Na]^+$ = 993.4537. FTIR (cm^{-1}): 3292 (νC–H alkyne), 3031 (νC–HAr), 2856 (νC–H alkyl), 2360 (νC≡C), 1975 (νC≡C–H), 1105 (νC–O), 700 (δC–H alkyne). $[\alpha_D] = 7.95$, $C = 1.51$ mg mL^{-1} in $CHCl_3$.

2,3,4-Tri-O-*benzyl-5*-O-*propargyl-6*-O-*trityl-D-glucitol (**6**).* To a solution of (**5**) (0.50 g, 0.52 mmol) in THF (18 mL) was added a TBAF (1.04 mL, 1.0 mmol, in THF C ~ 1 M) dropwise. The reaction mixture was heated at reflux for 4 hours, cooled and diluted with water (20 mL). The aqueous mixture was extracted with EtOAc (3 × 10 mL). The organic extracts were then washed with water (3 × 5 mL), brine (5 mL) and dried over $MgSO_4$. The dried extract was concentrated under reduced pressure to give a yellow oil which was purified by silica column chromatography (Hex/EtOAc/TEA: 9/1.9/0.1 v/v/v) to give (**6**) (0.28 g, 74%) as a colourless oil. ^1H (500 MHz $CDCl_3$) δ 7.46–7.44 (6H, m, HAr), 7.32–7.21 (22H, m, H_{Ar}), 7.07–7.05 (2H, m, H_{Ar}), 4.71 (1H, d, J = 11.4 Hz, CH_2–Ph), 4.67 (1H, d, J = 11.4 Hz, CH_2–Ph), 4.62 (1H, d, J = 11.4 Hz, CH_2–Ph), 4.56 (1H, d, J = 11.4 Hz, CH_2–Ph), 4.55 (2H, d, J = 11.4 Hz, CH_2–Ph), 4.31 (1H, dd, J = 2.3 Hz, 15.6 Hz, CH_2–alkyne), 4.13 (1H, dd, J = 2.3 Hz, 15.6 Hz, CH_2–alkyne), 3.99–3.97 (1H, m, H2), 3.95–3.92 (1H, m, H5), 3.89–3.86 (1H, m, H3), 3.77–3.72 (1H, m, H1), 3.78 (1H, qd, J = 4.9 Hz, H4), 3.63–3.59 (1H, dd, J = 4.9 Hz, 10.1 Hz, H1), 3.49 (1H, dd, J = 3.1 Hz, 10.5 Hz, H6), 3.34 (1H, dd, J = 5.5 Hz, 10.5 Hz, H6), 2.36 (1H, t, J = 2.3 Hz, CH≡C). ^{13}C (125 MHz $CDCl_3$) δ 143.8(3) (C_{Ar} Tr), 138.3, 138.3, 138.2 (C_{Ar} Bn), 128.7, 128.3, 128.2, 128.0(2), 127.9(2), 127.7, 127.6, 127.5, 127.3, 126.6 (CH_{Ar}), 86.9 ($C(Ph)_3$), 80.7 (C≡CH), 79.2 (C2), 79.0 (C5), 78.5 (C3), 78.2 (C4), 74.9 (CH_2–Ph), 74.8 (C≡CH), 75.8, 73.5 (CH_2–Ph), 62.9 (C6), 61.9 (C1), 57.7 (CH_2–alkyne). HRMS ES$^+$-TOF: Calculated for $C_{49}H_{48}O_6Na$ $[M + Na]^+$ = 755.3348, found $[M + Na]^+$ = 755.3349. FTIR (cm^{-1}): 3447 (νO–H), 3032, 3029 (νC–

HAr), 2885 (νC–H alkyl), 2361 (νC≡C), 1956 (νC≡C–H), 1090 (νC–O), 746 (δCsp^3–H), 699 (δC–H alkyne).

2,3,4-Tri-O-benzyl-1-O-(dibenzylphosphatyl)-5-O-propargyl-6-O-trityl-D-glucitol (7). Using procedure A, (**6**) (0.25 g, 0.34 mmol) was phosphorylated and purified by silica column chromatography (Hex/EtOAc/TEA: 9/0.9/0.1–7/2.9/0.1 v/v/v) to give (**7**) (0.27 g, 80%) as a colourless oil. ^{1}H (500 MHz $CDCl_3$) δ 7.47–1.46 (7H, m, H_{Ar}), 7.32–7.15 (31H, m, H_{Ar}), 6.99–6.97 (2H, m, H_{Ar}), 4.99–4.96 (4H, m, CH_2–OP), 4.68–4.45 (6H, m, CH_2–Ph), 4.35–4.33 (1H, m, H5), 4.30 (1H, dd, *J* = 2.4 Hz, 15.6 Hz, CH_2–alkyne), 4.22–4.09 (2H, m, H2, CH_2–alkyne), 4.01–3.99 (1H, m, H3), 3.94–3.87 (3H, m, H4, H1), 3.52 (1H, dd, *J* = 2.6 Hz, 10.5 Hz, H6), 3.31 (1H, dd, *J* = 5.1 Hz, 10.5 Hz, H6), 2.33 (1H, t, *J* = 2.3 Hz, CH≡C). ^{13}C (125 MHz $CDCl_3$) δ 143.8(3) (C_{Ar} Tr), 138.3, 138.2, 138.1 (C_{Ar} Bn), 135.8 (d, *J* = 7.1 Hz, C_{Ar}), 133.7 (d, *J* = 5.3 Hz, C_{Ar}), 128.7, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8(2), 127.7, 127.5, 127.4, 127.2, 126.9 (CH_{Ar}), 86.8 ($C(Ph)_3$), 80.1 (C≡CH), 78.6 (C5), 78.4 (d, *J* = 7.7 Hz, C2), 77.9 (C3), 77.6 (C4), 74.5 (CH≡C), 74.1, 73.5, 73.2 (CH_2–Ph), 69.1, (d, *J* = 5.8 Hz, CH_2–OP), 68.2 (d, *J* = 5.8 Hz, CH_2–OP), 64.7 (d, *J* = 5.4 Hz, C1), 63.0 (C6), 57.7 (CH_2–alkyne). ^{31}P (77 MHz $CDCl_3$) δ −0.53 (s, decoupled). HRMS ES$^+$-TOF: Calculated for $C_{63}H_{61}O_9PNa$ $[M + Na]^+$ = 1014.4366, found $[M + Na]^+$ = 1014.4355. $[\alpha_D]$ = −4.49, *C* = 0.89 mg mL^{-1} in $CHCl_3$.

2,3,4-Tri-O-benzyl-1-O-tert-butyldiphenylsilyl-5-O-propargyl-D-glucitol (8). To a solution of (**5**) (0.040 g, 0.041 mmol) in Et_2O (2 mL) was carefully added aluminium trichloride powder (0.016 mg, 0.12 mmol) portion-wise. After 5 hours at rt, the reaction mixture was diluted with Et_2O (5 mL) and carefully quenched with water (5 mL). The organic phase was washed with a saturated $NaHCO_3$ solution (5 mL), water (3 × 5 mL), brine (5 mL) and was dried over $MgSO_4$. The dried filtrate was concentrated under reduced pressure to give a crude yellow oil. This residue was purified by silica column chromatography (Hex/EtOAc: 75/25 v/v) to give (**8**) (0.020 mg, 67%) as colourless oil. ^{1}H (500 MHz $CDCl_3$) δ 7.69–7.60 (4H, m, H_{Ar}), 7.47–7.23 (21H, m, H_{Ar}), 4.73–4.69 (2H, m, CH_2–Ph), 4.60 (1H, d, *J* = 11.4 Hz, CH_2–Ph), 4.54 (1H, d, *J* = 11.4 Hz, CH_2–Ph), 4.02 (2H, dd, *J* = 2.3 Hz, 16.0 Hz, CH_2–alkyne), 3.96–3.92 (2H, m, H6, H5), 3.88–3.73 (6H, m, H6, H1, H2, H4), 3.69 (1H, quadruplet, *J* = 4.3 Hz, H3), 2.32 (1H, t, *J* = 2.3 Hz, CH≡C), 2.28 (1H, t, *J* = 6.3 Hz, HO), 1.08 (9H, s, $C(CH_3)_3$). ^{13}C (125 MHz $CDCl_3$) δ 138.5, 138.3, 138.2 (C_{Ar} Bn), 135.7, 135.6 (CH_{Ar} TBDPS), 133.4, 133.3 (C_{Ar} TBDPS), 130.4, 130.35 (CH_{Ar} TBDPS), 129.8, 129.7, 129.6, 128.6, 128.4, 128.3, 128.2, 128.0, 127.9(2), 127.8, 127.7, 127.5, 127.3 (CH_{Ar}), 80.0 (C2), 79.9 (C≡CH), 79.7 (C5), 78.9 (C3), 78.4 (C4), 74.7 (CH_2–Ph), 74.6 (CH≡C), 75.5, 73.0 (CH_2–Ph), 63.3 (C1), 60.9 (C6), 56.8 (CH_2–alkyne), 26.7 (CH_3), 19.1 ($C(CH_3)_3$). HRMS ES$^+$-TOF: Calculated for $C_{46}H_{52}O_6SiNa$ $[M + Na]^+$ = 751.3434, found $[M + Na]^+$ = 751.3431. FTIR (cm^{-1}): 3440 (νO–H), 3033, 3029 (νC–HAr), 2886 (νC–H alkyl), 2360 (νC≡C), 1963 (νC≡C–H), 1089 (νC–O), 745 (δCsp^3–H), 698 (δC–H alkyne).

2,3,4-Tri-O-benzyl-6-O-(dibenzylphosphatyl)-1-O-tert-butyldiphenylsilyl-5-O-propargyl-D-glucitol (9). Using procedure A, (**8**) (1.00 g, 1.4 mmol) was phosphorylated and purified by silica column chromatography (Hex/EtOAc: 9/1–7/3 v/v) to give (**9**) (1.00 g, 74%) as a colourless oil. ^{1}H (500 MHz $CDCl_3$) δ 7.69–7.65 (5H, m, H_{Ar}), 7.42–7.16 (30H, m, H_{Ar}), 5.07–5.03 (4H, m, CH_2–OP), 4.73–4.53 (6H, m, CH_2–Ph), 4.48–4.44 (1H, m, H5), 4.25–4.20 (1H, m, H2), 4.15 (1H, dd, *J* = 2.4 Hz, 15.8 Hz, CH_2–alkyne), 4.04 (1H, dd, *J* = 2.4 Hz, 15.8 Hz, CH_2–alkyne), 3.95 (1H, dd, *J* = 3.3 Hz, 10.7 Hz, H1), 3.92–3.88 (3H, m, H1, H6), 3.86–3.83 (1H, m, H4), 3.82–3.78 (1H, m, H3), 2.23 (1H, t, *J* = 2.4 Hz, CH≡C), 1.08 (9H, s, $C(CH_3)_3$). ^{13}C (125 MHz $CDCl_3$) δ 138.5, 138.3, 138.0 (C_{Ar} Bn), 135.7 (d, *J* = 6.9 Hz, C_{Ar}), 135.5 (CH_{Ar} TBDPS), 133.3, 133.2 (C_{Ar} TBDPS), 133.1 (d, *J* = 5.9 Hz, C_{Ar}), 129.5(2) (CH_{Ar} TBDPS), 128.4, 128.3, 128.1(2), 128.0(2), 127.8(2), 127.7, 127.6(2), 127.4, 127.0 (CH_{Ar}), 80.2 (C2), 79.6 (C≡CH), 78.3 (d, *J* = 7.3 Hz, C5), 78.2 (C4), 77.8 (C3), 74.3, 73.9, 73.0 (CH_2–Ph), 69.8 (CH≡C), 69.1 (d, *J* = 5.5 Hz, CH_2–OP), 66.8 (d, *J* = 5.5 Hz, C6), 63.7 (C1), 57.2 (CH_2–alkyne), 26.7 (CH_3), 19.0 ($C(CH_3)_3$). ^{31}P (77 MHz $CDCl_3$) δ −0.01 (s, decoupled). HRMS ES$^+$-TOF: Calculated for $C_{60}H_{65}O_9SiPNa$ $[M + Na]^+$ = 1011.4033, found $[M + Na]^+$ = 1011.4040. FTIR (cm^{-1}): 3447 (νO–H), 3063, 3030 (νC–HAr), 2856 (νC–H alkyl), 2360 (νC≡C), 1958 (νC≡C–H), 1112 (νP–O–C), 1071 (νC–O), 741 (δCsp^3–H), 699 (δC–H alkyne).

Azido-2,3-isopropylidene-β-D-ribofuranoside (12). To a suspension of (**11**) (4.00 g, 8.2 mmol) in methanol (50 mL) at rt, MeONa (1.77 g, 32.8 mmol, 25% wt in MeOH) was added dropwise. After being stirred for 3 hours at rt, the reaction mixture was neutralised by successive filtrations through a pad of Dowex (R) HCR-W2 until pH 7. Methanol was then removed under reduced pressure and the residue was dissolved in acetone (50 mL) and 2,2-dimethoxypropane (0.94 g, 9.0 mmol) was added dropwise to the solution. A catalytic amount of concentrated sulfuric acid (0.1 mL) was added and the reaction was stirred for a further 4 hours. The reaction was quenched with a saturated $NaHCO_3$ solution (50 mL). The acetone was then removed under reduced pressure and the residue was diluted with water (100 mL). The aqueous solution was extracted with EtOAc (3 × 50 mL), and the combined extracts were washed with water (*n* × 25 mL) until neutral pH and then with brine (25 mL). The organic solution was dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by column chromatography (Hex/EtOAc: 9/1–75/25) to give (**12**) (1.50 g, 87% overall) as a colourless oil.

^{1}H (500 MHz, $CDCl_3$), δ (ppm) 5.54 (1H, m, H1), 4.77 (1H, dd, *J* = 0.8 Hz, 5.9 Hz, H2), 4.53 (1H, d, *J* = 5.9 Hz, H3), 4.41 (1H, m, H4), 3.79–3.75 (1H, m, H5), 3.71–3.66 (1H, m, H5), 2.33–2.30 (1H, m, OH), 1.49 (3H, s, CH_3), 1.32 (3H, s, CH_3). ^{13}C (500 MHz, $CDCl_3$), δ (ppm) 113.4 (C isopropylidene), 98.5 (C1), 89.0 (C4), 86.3 (C3), 82.1 (C2), 64.1 (C5), 26.9, 25.3 (CH_3). HRMS ES$^+$-TOF: Calculated for $C_8H_{14}N_3O_4$ $[M + H]^+$ = 216.0984, found $[M + H]^+$ = 216.0982.

Azido-2,3-O-acetyl-5-O-(dibenzylphosphate)-β-D-ribofuranoside (14). Using procedure A, (**12**) (1.44 g, 6.7 mmol) was phosphorylated and then purified by flash chromatography (Hex/EtOAc 75/25) to give azido-2,3-isopropylidene-5-(dibenzylphosphate)-β-D-ribofuranoside. The collected fractions containing

the desired product were concentrated and dissolved in a mixture of TFA–water (50 mL, 8 : 2 v/v) and stirred for 8 hours at rt. The reaction mixture was then extracted with EtOAc (3 × 10 mL). The combined organic extracts were washed with an aqueous saturated solution of $NaHCO_3$ until pH over 7, then with water (until neutral pH), brine (10 mL) and then dried over $MgSO_4$. The organic layer was concentrated under reduced pressure and the crude material was purified by column chromatography (Hex/EtOAc: 75/25–0/1) to give the corresponding diol intermediate (1.95 g, 67% overall) as colourless oil.

^{1}H (500 MHz, $CDCl_3$), δ (ppm) 7.28–7.26 (10H, s, H_{Ar}), 5.27 (1H, d, J = 1.8 Hz, H1), 5.20 (1H, dd, J = 4.9 Hz, 6.0 Hz, H3), 5.04 (1H, dd, J = 1.8 Hz, 4.9 Hz, H2), 4.99 (4H, m, CH_2–Ph), 4.22–4.20 (1H, m, H4), 4.13–4.09 (1H, m, H5), 4.04–4.01 (1H, m, H5). ^{13}C (125 MHz, $CDCl_3$), δ (ppm) 135.9 (d, J = 5.9 Hz, C_{Ar}), 135.6 (d, J = 5.4 Hz, C_{Ar}), 128.6, 127.9, 127.8(2) (CH_{Ar}), 93.4 (C1), 78.5 (d, J = 8.3 Hz, C4), 72.4 (C2), 68.2 (C3), 69.4, 67.9 (d, J = 5.5 Hz, CH_2–Ph,), 66.2 (d, J = 5.5 Hz, C5). ^{31}P (77 MHz, $CDCl_3$), δ (ppm) −0.68 (s, decoupled). HRMS ES$^+$-TOF: Calculated for $C_{17}H_{18}N_3O_7P$ $[M + H]^+$ = 436.1274, found $[M + H]^+$ = 436.1270. FTIR (cm^{-1}): 3031 (νC–HAr), 2946 (νC–H alkyl), 2112 (νN_3), 1456 (νC–O), 1246 (νC–N), 1020 (νP–O–C), 739 (νCsp^3–H). $[\alpha_D]$ = −104.7, (c 0.42 mg mL^{-1} $CHCl_3$).

This pure product was then acetylated using acetic anhydride (0.6 mL, 6.18 mmol) in pyridine (50 mL) and the reaction mixture was stirred for 6 hours at rt. The pyridine was partially removed *in vacuo*. The residue was dissolved in EtOAc (50 mL) and the organic layer was washed with a saturated copper sulfate solution until the blue colour remained unchanged. The organic solution was washed with water (3 × 20 mL), brine (20 mL) and dried over $MgSO_4$. The organic solution was concentrated under reduced pressure and the crude material was purified by silica column chromatography (Hex/EtOAc: 75/25 v/v) to give (**14**) (1.05 g, 80%) as a waxy colourless oil.

^{1}H (500 MHz, $CDCl_3$) δ 7.27–7.25 (10H, m, HAr), 5.28 (1H, d, J = 1.8 Hz, H1), 5.21 (1H, dd, J = 4.9 Hz, 6.0 Hz, H3), 5.03 (1H, dd, J = 1.8 Hz, 4.9 Hz, H2), 4.98 (4H, m, CH_2–Ph), 4.22–4.19 (1H, m, H4), 4.12–4.08 (1H, m, H5), 4.04–4.00 (1H, m, H5), 2.02 (3H, s, CH_3), 1.94 (3H, s, CH_3). ^{13}C (125 MHz, $CDCl_3$) δ 169.3, 169.2 (C═O Ac), 135.9 (d, J = 6.7 Hz, C_{Ar}), 135.6 (d, J = 4.9 Hz, C_{Ar}), 128.6, 127.9, 127.8(2) (CH_{Ar}), 92.9 (C1), 79.9 (d, J = 8.3 Hz, C4), 74.4 (C2), 70.2 (C3), 69.4, 68.5 (d, J = 5.5 Hz, CH_2–Ph), 66.2 (d, J = 5.5 Hz, C5), 20.4, 20.3 (CH_3). ^{31}P (77 MHz, $CDCl_3$) δ −0.36 (s, decoupled). HRMS ES$^+$-TOF: Calculated for $C_{24}H_{22}N_3O_9PNa$ $[M + Na]^+$ = 542.1304, found $[M + Na]^+$ = 542.1356. $[\alpha_D]$ = −69.1 (c 0.68 mg mL^{-1} $CHCl_3$).

*1-[5-*O*-(Dibenzylphosphatyl)-β-D-ribofuranosyl]-4-methylenoxy-[5-(2*R*,3*S*,4*R*,5*R*)-2,3,4-tris-*O*-benzyl-1-*O*-(*tert*-butyldiphenylsilyl)-6-*O*-trityl-hexanyl]-1*H*-1,2,3-triazole (**16**).* Using procedure B, (**5**) (0.051 g, 0.052 mmol) was mixed with (**14**) (0.030 g, 0.057 mmol) in a water–*t*BuOH (1 mL) mixture. A pre-mixed copper sulfate (0.006 g, 0.021 mmol), sodium ascorbate (0.008 mg, 0.042 mmol) aqueous solution (1 mL) was added to the previous suspension. After 6 hours and removal under vacuum of the solvents, the crude material was dissolved in MeOH (3 mL) and MeONa (0.1 mL 25% wt in MeOH). The reaction mixture was stirred for 20 minutes and concentrated. The crude was then purified by silica column chromatography (Hex/EtOAc/TEA: 8/1.9/0.1–0/0.9/0.1 v/v/v) to give (**16**) (0.026 g, 37% over 2 steps) as a colourless oil. ^{1}H (500 MHz $CDCl_3$) δ 7.70 (1H, s, H triazole), 7.66–7.60 (5H, m, H_{Ar}), 7.43–7.20 (43H, m, H_{Ar}), 7.00–6.97 (2H, m, H_{Ar}), 5.81 (d, J = 1.8 Hz, H1′), 5.11–5.02 (4H, m, CH_2–OP), 4.85 (1H, d, J = 11.5 Hz, CH_2–Ph), 4.78 (1H, d, J = 11.8 Hz, CH_2–triazole), 4.65–4.63 (5H, m, CH_2–Ph), 4.62 (1H, d, J = 11.8 Hz, CH_2–triazole), 4.51 (2H, m, H5′), 4.30–4.25 (1H, m, H4′), 4.20–4.17 (1H, m, H2′), 4.05–4.01 (1H, m, H5), 4.00–3.95 (5H, m, H1, H3, H4, H3′), 3.50 (1H, dd, J = 2.4 Hz, 10.5 Hz, H6), 3.30 (1H, dd, J = 5.6 Hz, 10.5 Hz, H6), 1.04 (9H, s, $C(CH_3)_3$). ^{13}C (125 MHz $CDCl_3$) δ 46.2 (C triazole) 143.3(3) (C_{Ar} Tr), 137.6(2), 136.7 (C_{Ar} Bn), 135.6 (CH_{Ar}), 135.5 (d, J = 7.0 Hz, C_{Ar}), 133.4 (d, J = 3.3 Hz, C_{Ar}), 130.1, 129.4, 128.5, 128.3, 128.2, 128.1, 127.9, 127.8(2), 127.7(2), 127.6, 127.5, 127.4 (CH_{Ar}), 122.7 (CH triazole), 89.8 (C1′), 86.7 ($C(Ph)_3$), 81.3 (d, J = 7.6 Hz, C4′), 80.0 (C4), 78.6 (C2), 77.7 (C5), 77.3 (C3), 74.0, 73.6, 73.4 (CH_2–Ph), 72.3 (C2′), 69.5(2) (d, J = 3.7 Hz, CH_2–OP), 68.2 (C3′), 66.1 (d, J = 6.1 Hz, C5′), 64.7 (CH_2–triazole), 63.6 (C6), 63.0 (C1), 26.0 $(CH_3)_3$, 19.1 $(C(CH_3)_3)$. ^{31}P (77 MHz $CDCl_3$) δ −0.11 (s, decoupled). HMRS ES$^+$-TOF: Calculated for $C_{84}H_{88}N_3O_{13}PSiNa$ $[M + Na]^+$ = 1428.5728, $[M + Na]^+$ = 1428.5728.

*1-[(2,3-Di-*O*-Acetyl-5-*O*-dibenzylphosphatyl)-β-D-ribofuranosyl]-4-methylenoxy-[5-(2*R*,3*S*,4*R*,5*R*)-2,3,4-tris-*O*-benzyl-1-*O*-(dibenzyl-phosphatyl)-6-*O*-trityl-hexanyl]-1*H*-1,2,3-triazole (**17**).* Using procedure B, alkyne (**7**) (0.046 g, 0.046 mmol) was mixed with (**14**) (0.026 g, 0.051 mmol) in a water–*t*BuOH (1 mL) mixture. A pre-mixed copper sulfate (0.005 g, 0.022 mmol), sodium ascorbate (0.007 g, 0.044 mmol) aqueous solution (1 mL) was added to the suspension. After 8 hours and removal under vacuum of the solvents, the crude material was purified by silica column chromatography (Hex/EtOAc/TEA: 1/0.9/0.1–0/0.9/0.1 v/v/v) to give **13** (0.047 g, 73%) as a colourless oil. ^{1}H (500 MHz $CDCl_3$) δ 7.71 (1H, s, H triazole), 7.44–7.42 (6H, m, H_{Ar}), 7.28–7.11 (42H, m, H_{Ar}), 6.93–6.92 (2H, m, H_{Ar}), 5.05 (1H, d, J = 3.8 Hz, H1′), 5.78 (1H, dd, J = 3.8 Hz, 5,2 Hz, H2′), 5.66 (1H, t, J = 5.2 Hz, H3′), 5.00–4.91(8H, m, CH_2–OP), 4.78 (1H, d, J = 11.9 Hz, CH_2–triazole), 4.63–4.40 (8H, m, CH_2–Ph, H4′, CH_2–triazole), 4.33–4.29 (1H, m, H1), 4.22–4.10 (3H, m, H1, H5′), 3.98–3.96 (1H, m, H3), 3.90–3.87 (1H, m, H5), 3.86–3.81 (2H, m, H4, H2), 3.60 (1H, dd, J = 2,7 Hz, 10.5 Hz H6), 3.32 (1H, dd, J = 5.2 Hz, 10.5 Hz H6), 2.09 (3H, s, CH_3), 2.06 (3H, s, CH_3). ^{13}C (125 MHz $CDCl_3$) δ 169.2, 169.0 (C═O Ac), 145.7 (C triazole), 143.8(3) (C_{Ar} Tr), 138.2, 138.1, 138.0 (C_{Ar}), 135.8(2), 135.4 (2) (C_{Ar}, d, J = 6.5 Hz), 128.7, 128.5(2), 128.4, 128.2(2), 128.0(2), 127.9(2), 127.8, 127.5(2), 127.3, 127.0(2) (CH_{Ar}), 122.3 (CH triazole), 89.7 (C1′), 86.8 (CPh_3), 81.2 (d, J = 7.6 Hz, C4′), 79.6 (C4), 78.2 (d, J = 7.6 Hz, C2), 77.7 (C5), 77.2 (C3), 74.1 (C2′), 74.0, 73.4, 73.1 (CH_2–Ph), 70.7 (C3′), 69.5(2) (d, J = 3.7 Hz, CH_2–OP), 68.2 (d, J = 2.1 Hz, CH_2–OP), 68.2 (d, J = 6.1 Hz, C5′), 66.4 (d, J = 5.1 Hz, C1), 64.1 (CH_2–triazole), 63.0 (C6), 20.4, 20.3 (CH_3). ^{31}P (77 MHz $CDCl_3$) δ 0.19 (s, decoupled), −0.09 (s, decoupled). LRMS: Calculated MALDI-TOF: Calculated for

$C_{86}H_{87}N_3O_{18}P_2Na$ $[M + Na]^+$ = 1534.5, found $[M + Na]^+$ = 1534.3.

*1-[(2,3,5-Tri-*O*-benzoyl)-β-D-ribofuranosyl]-4-methylenoxy-[5-(2*R*,3*S*,4*R*,5*R*)-2,3,4-tris-*O*-benzyl-6-*O*-(dibenzylphosphatyl)-1-*O*-(tert-butyldiphenylsilyl))-hexanyl]-1*H*-1,2,3-triazole **(18)**.* Using procedure B, (**9**) (0.043 g, 0.043 mmol) was mixed with (**11**) (0.023 g, 0.047 mmol) in a water–*t*BuOH (1 mL) mixture. A pre-mixed copper sulfate (0.004 g, 0.022 mmol), sodium ascorbate (0.007 g, 0.044 mmol) aqueous solution (1 mL) was added to the previous suspension. After 12 hours and removal under vacuum of the solvents, the crude material was purified by silica column chromatography (Hex/EtOAc: 1/1–0/1 v/v) to give (**18**) (0.037 g, 58%) as a colourless oil. ^{1}H (500 MHz $CDCl_3$) δ 8.02–7.94 (6H, m, H_{Ar}), 7.68 (1H, s, H triazole), 7.66–7.12 (44H, m, H_{Ar}), 6.25 (1H, dd, J = 2.8 Hz, 5.2 Hz, H2′), 6.26 (1H, t, J = 6.3 Hz, H3′), 6.16 (1H, d, J = 2.8 Hz, H1′), 5.00–4.98 (4H, m, CH_2–OP), 4.87–4.84 (1H, m, H4′), 4.73–4.42 (10H, CH_2–Ph, CH_2–triazole, H5, H4), 4.24–4.19 (1H, m, H5′), 3.98–3.78 (H6, H1, H2, H3), 1.04 (9H, s, $C(CH_3)_3$). ^{13}C (125 MHz $CDCl_3$) δ 166.0, 165.0, 164.8 (C═O Bz), 145.7 (C triazole), 138.5, 138.3, 138.1 (C_{Ar} Bn), 135.8 (d, J = 7.0 Hz, C_{Ar}), 135.6 (CH_{Ar}), 133.3 (d, J = 3.4 Hz, C_{Ar}), 129.8, 129.7, 129.6(2) (CH_{Ar}), 129.3, 128.7, 128.6 (C_{Ar}), 128.5(2), 128.4 (CH_{Ar}), 128.4(2), 128.2 (C_{Ar} Bz), 128.1, 127.8(2), 127.7, 127.6(2), 127.5, 127.4 (CH_{Ar}), 122.7 (CH triazole), 90.1 (C1′), 80.8 (C4′), 80.2 (C4), 79.5 (d, J = 6.9 Hz, C5), 78.2 (C2), 77.9 (C3), 75.2 (C2′), 74.3, 73.9, 73.0 (CH_2–Ph), 71.8 (C5′), 69.1(2) (d, J = 3.1 Hz, CH_2–OP), 67.2 (d, J = 5.9 Hz, C5), 63.9 (CH_2–triazole), 63.8 (C6), 26.9 (CH_3), 19.1 ($C(CH_3)_3$). ^{31}P (77 MHz $CDCl_3$) δ 0.18 (s, decoupled). HRMS: Calculated ES$^+$-TOF: Calculated for $C_{86}H_{87}N_3O_{16}P_2Na$ $[M + H]^+$ = 1476.5593, found $[M + H]^+$ = 1476.5659.

*1-[2,3-Di-*O*-acetyl-5-*O*-(dibenzylphosphatyl)-β-D-ribofuranosyl]-4-methylenoxy-[5-(2*R*,3*S*,4*R*,5*R*)-2,3,4-tris-*O*-benzyl-6-*O*-(dibenzylphosphatyl)-1-*O*-(tert-butyldiphenylsilyl))-hexanyl]-1*H*-1,2,3-triazole **(19)**.* Using procedure B, alkyne (**9**) (0.055 g, 0.055 mmol) was mixed with (**14**) (0.032 g, 0.061 mmol) in a water–*t*BuOH (1 mL) mixture. A pre-mixed copper sulfate (0.006 g, 0.021 mmol), sodium ascorbate (0.009 g, 0.042 mmol) aqueous solution (1 mL) was added to the previous mixture. After 8 hours and removal under vacuum of the solvents, the crude material was purified by silica column chromatography (Hex/EtOAc: 1/1–0/1 v/v) to give (**19**) (0.051 g, 62%) as a colourless oil. ^{1}H (500 MHz $CDCl_3$) δ 7.66–7.61 (5H, H triazole, H_{Ar}), 7.40–7.14 (38H. m, H_{Ar}), 7.11–7.09 (2H, m, H_{Ar}), 5.93 (1H. d, J = 3.8 Hz, H1′), 5.76 (1H, dd, J = 3.8 Hz, 5.2 Hz, H2′), 5.64 (1H, t, J = 5.2 Hz, H3′), 5.02–4.95 (4H, m, CH_2–OP), 4.67 (1H. d, J = 11.8 Hz, CH_2–Ph), 4.64–4.38 (10H, m, H5′, H4′, CH_2–triazole, CH_2–Ph), 4.21–4.14 (2H, m, H6), 3.95 (1H, dd, J = 2.9 Hz, 10.8 Hz, H1), 3.87–3.77 (5H, m, H1, H2, H3, H4, H5), 2.08 (3H, s, CH3), 20.6 (3H, s, CH_3), 1.02 (9H, s, $C(CH_3)_3$). ^{13}C (125 MHz $CDCl_3$) δ 169.2, 168.9 (C═O, Ac), 145.5 (C triazole), 138.6, 138.3, 138.0 (C_{Ar} Bn), 135.9(2) (d, J = 7.0 Hz, C_{Ar}), 135.6 (CH_{Ar}), 133.3(2) (d, J = 3.3 Hz, C_{Ar}), 129.6(2), 128.5(2), 128.3(2), 128.2, 128.1(2), 127.9(2), 127.8(3), 127.7, 127.6, 127.5, 127.4(2) (CH_{Ar}), 122.6 (CH triazole), 89.7 (C1′), 81.2 (d, J = 7.6 Hz, C4′), 80.2 (C4), 79.5 (d, J = 7.1 Hz, C5), 78.2 (C2), 77.9 (C3), 74.3, 73.9, 73.0 (CH_2–Ph), 74.2 (C2′), 70.7 (C3′), 69.5, 69.1 (CH_2–OP), 67.2 (d, J = 5.9Hz, C5′), 66.4 (d, J = 5.2 Hz, C6), 63.9 (CH_2–Triazole), 63.7 (C1), 26.1 (CH_3), 20.4, 20.3 (CH_3), 19.1 ($C(CH3)_3$). ^{31}P (77 MHz $CDCl_3$) δ 0.17 (s, decoupled), 0.01 (s, decoupled). LRMS MALDI-TOF: Calculated $C_{83}H_{91}N_3O_{18}P_2SiNa$ $[M + Na]^+$ = 1530.5, found $[M + Na]^+$ = 1530.4.

*1-[(5-*O*-Phosphatyl)-β-D-ribofuranosyl]-4-methylenoxy-[5-(2*S*,3*R*,-4*R*,5*R*)-hexan-1,2,3,4,6-pentaolyl]-1*H*-1,2,3-triazole **(23)**.* Using procedure D, compound (**16**) (48 mg, 0.034 mmol) was deprotected and purified to give (**23**) (14 mg, 84% overall) as a white gum. ^{1}H (500 MHz D_2O) δ 8.20 (1H, s, H triazole), 5.98 (1H, d, J = 5.0 Hz, H1′), 4.47 (1H, t, J = 4.5 Hz, H2′), 4.27 (1H, t, J = 4.5 Hz, H3′), 4.19–4.16 (1H, m, H4′), 3.88–3.80 (3H, m, H5′, H5), 3.74–3.70 (1H, d, J = 12.1 Hz, CH_2–triazole), 3.61–3.47 (7H, m, H6, CH_2–triazole, H2, H3, H4, H1), 3.38 (1H, dd, J = 6.6 Hz, 11.7 Hz, H6). ^{13}C (125 MHz D_2O) δ 143.6 (C triazole), 123.1 (CH triazole), 92.1 (C1′), 84.7 (d, J = 5.7 Hz, C4′), 78.9 (C5), 75.2 (C4), 72.8 (C3), 70.5 (C2′), 69.5 (C2), 69.4 (C3′), 64.1 (d, J = 4.3 Hz, C5′), 62.2 (C6), 62.2 (C1), 59.4 (CH_2–triazole). ^{31}P (77 MHz D_2O) δ 2.08 (s, decoupled). HRMS ES$^-$-TOF: Calculated for $C_{14}H_{25}N_3O_{13}P$ $[M - H]^-$ = 474.1125, found $[M - H]^-$ = 474.1110.

*1-[(5-*O*-Phosphatyl)-β-D-ribofuranosyl]-4-methylenoxy-[5-(2*S*,3*R*,-4*R*,5*R*)-1-*O*-(phosphatyl)-hexan-2,3,4,6-tetraolyl]-1*H*-1,2,3-triazole **(24)**.* Using procedure D, compound (**17**) (85 mg, 0.060 mmol) was deprotected purified to give (**24**) (31 mg, 94% overall) as a white gum. ^{1}H (500 MHz D_2O) δ 8.22 (1H, s, H triazole), 6.10 (1H, d, J = 4.5 Hz, H1′), 4.66 (1H, t, J = 4.8 Hz, H2′), 4.30 (1H, t, J = 4.8 Hz, H3′), 4.20–4.18 (1H, m, H4′) 4.06–3.65 (12H, m, H1, H2, H3, H4, H5, H6, H5′,CH_2–triazole). ^{13}C (125 MHz D_2O) δ 144.5 (C triazole), 123.6 (CH triazole), 92.1 (C1′), 84.7 (d, J = 7.2 Hz, C4′), 77.9 (C5), 75.5 (C4), 73.4 (C3), 71.7 (d, J = 6.2 Hz, C2), 70.9 (C2′), 69.6 (C3′), 65.2 (d, J = 4.7 Hz, C5′), 63.4 (d, J = 5.0 Hz, C1), 62.8 (C6), 62.7 (CH_2–triazole). ^{31}P (77 MHz D_2O) δ 5.68 (s, decoupled), 5.30 (s, decoupled). HRMS: Calculated ES$^-$-TOF for $C_{14}H_{26}N_3O_{16}P_2$ $[M - H]^-$ = 554.0788, found $[M - H]^-$ = 554.0878.

*1-(β-D-Ribofuranosyl)-4-methylenoxy-[5-(2*S*,3*R*,4*R*,5*R*)-6-*O*-phosphatyl-hexan-1,2,3,4-tetraolyl]-1*H*-1,2,3-triazole **(21)**.* Using procedure C, compound (**18**) (120 mg, 0.078 mmol) was deprotected and purified to give compound (**21**) (30 mg, 81% overall) as a white gum. ^{1}H (500 MHz D_2O) δ 8.19 (1H, s, H triazole), 6.05 (1H, dd, J = 1.7 Hz, 3.9 Hz, H1′), 4.59 (1H, dt, J = 1.7 Hz, 5.0 Hz, H2′), 4.33 (1H, dt, J = 1.7 Hz, 5.0 Hz, H3′), 4.19–4.13 (2H, m, CH_2–triazole, H4′), 4.00–3.96 (2H, m, CH_2–triazole, H5), 3.77–3.57 (8H, m, H1, H2, H3, H4, H5′, H6), 3.50–3.46 (1H, ddd, J = 1.7 Hz, 6.6 Hz, 11.7 Hz, H6). ^{13}C (125 MHz D_2O) δ (ppm): 144.0 (C triazole), 123.9 (CH triazole), 92.0 (C1′), 85.1 (C4′), 77.4 (d, J = 7.8 Hz, C5), 74.7 (C4), 72.8 (C3), 70.0 (C2′), 69.3 (C2), 69.0 (C3′), 63.7 (d, J = 4.9 Hz, C6), 62.2 (C1), 61.9 (C5′), 60.9 (CH_2–triazole). ^{31}P (77 MHz D_2O) δ 1.36 (broad, decoupled). HRMS ES$^-$-TOF: Calculated $C_{14}H_{25}N_3O_{13}P$ $[M - H]^-$ = 474.1125, found $[M - H]^-$ = 474.1135.

*1-[(5-*O*-Phosphatyl)-β-D-ribofuranosyl]-4-methylenoxy-[5-(2*S*,3*R*,-4*R*,5*R*)-6-*O*-phosphatyl-hexan-1,2,3,4-tetraolyl]-1*H*-1,2,3-triazole*

(22). Using procedure D, compound (**19**) (91 mg, 0.065 mmol) was deprotected and purified to yield (**22**) (27 mg, 73% overall) as a pure white gum. ^{1}H (500 MHz D_2O) δ 8.24 (1H, s, H triazole), 6.08 (1H, d, J = 4.6 Hz, H1′), 4.56 (1H, t, J = 4.7 Hz, H2′), 4.37 (1H, t, J = 4.7 Hz, H3′), 4.29–4.28 (1H, m, H4′), 4.18–4.14 (1H, m, H5), 4.06–4.02 (2H, m, H5′), 3.99–3.95 (2H, m, CH_2–triazole), 3.75–3.65 (5H, m, H1, H2, H3, H4), 3.59 (1H, dd, J = 3.06 Hz, 11.8 Hz, H6), 3.47 (1H, dd, J = 6.6 Hz, 11.8 Hz, H6). ^{13}C (125 MHz D_2O) δ 143.8 (C triazole), 123.9 (CH triazole), 92.8 (C1′), 84.6 (d, J = 8.6 Hz, C4′), 77.9 (d, J = 7.3 Hz, C5), 75.5 (C4), 73.4 (C3), 70.8 (C2′), 70.7 (C2), 69.7 (C3′), 69.4 (d, J = 7.7 Hz, C6), 65.0 (d, J = 4.5 Hz, C5′), 62.8 (C1), 62.7 (CH_2–triazole). ^{31}P (77 MHz D_2O) δ 1.20 (broad, decoupled). HRMS ES$^-$-TOF: Calculated for $C_{14}H_{26}N_3O_{16}P_2$ $[M - H]^-$ = 554.0788, found $[M - H]^-$ = 554.0775.

*3,4-Di-*O*-benzyl-1-*O*-*tert*-butyldiphenylsilyl-5-*O*-propargyl-6-*O*-trityl-2-deoxy-D-glucitol (30).* To a solution of (**29**) (0.12 g, 0.15 mmol) in dry THF (5 mL) was added sodium hydride (0.06 g, 0.15 mmol, 60% wt in oil) at rt. The reaction mixture was stirred for 20 minutes at rt and propargyl bromide (0.018 mL, 0.16 mmol, 80% wt in toluene) was added. After 8 hours at rt, the reaction mixture was diluted with water (10 mL) and the aqueous mixture was extracted with EtOAc (3 × 5 mL). The combined extracts were washed with water (3 × 5 mL), brine (5 mL) and dried over $MgSO_4$. EtOAc was removed under reduced pressure and the crude product was purified by silica column chromatography (Hex/EtOAc/TEA: 9/0.9/0.1 v/v/v) to yield (**30**) (0.097 g, 77%) as colourless oil. δ_H (500 MHz; $CDCl_3$; Me_4Si) 7.68–7.67 (4H, m, Ph), 7.46–7.35 (5H, m, Ph), 7.32–7.20 (24H, m Ph), 7.09–7.07 (2H, m, Ph), 4.60–4.45 (4H, m, CH_2–Ph), 4.21 (1H, d, J = 2.3 Hz, CH_2–alkyne), 4.16 (1H, d, J = 2.3 Hz, CH_2–alkyne), 4.02–3.87 (3H, m, H-5, H-1), 3.81–3.66 (2H, m, H-3, H-4), 3.54 (1H, dd, J = 2.4 Hz, 10.5 Hz, H-6), 3.34 (1H, dd, J = 5.6 Hz, 10.5 Hz, H-6), 2.36 (1H, t, J = 2.4 Hz, CH≡C), 1.87–1.81 (2H, m, H-2), 1.09 (9H, s, (CH_3)). δ_C (125 MHz; $CDCl_3$; Me_4Si) 143.9(3), 138.5, 138.3, 133.6, 133.5, 129.6, 128.7, 128.3(2), 128.1, 128.0(2), 127.9, 127.8, 127.6(2), 127.4, 127.0, 86.8, 80.8 (C-5), 80.0 (C≡CH), 79.5 (C-3), 76.4 (C-4), 74.5 (CH≡C), 74.2, 73.0 (CH_2–Ph), 63.1 (C-1), 60.4 (C-6), 57.3 (CH_2–alkyne), 33.2 (C-2), 26.8 (CH_3), 19.4 (C–$(CH_3)_3$). HRMS (ES) calculated $[M + Na]^+$ = 887.4108; found $[M + Na]^+$ = 887.4127. FTIR (cm^{-1}): 3294 (νC–H alkyne), 3030 (νC–H_{Ar}), 2857 (νC–H alkyl), 2361 (νC≡C), 1978 (νC≡C–H), 1100 (νC–O), 702 (νC–H alkyne). $[\alpha]_D^{22}$ +15.3 (c 0.39 mg mL^{-1} in $CHCl_3$).

*3,4-Di-*O*-benzyl-1-*O*-*tert*-butyldiphenylsilyl-5-*O*-propargyl-2-deoxy-D-glucitol (31).* To a solution of (**30**) (0.12 g, 0.14 mmol) in Et_2O (20 mL) was added aluminium trichloride (0.036 g, 0.27 mmol) portion-wise at rt. The reaction was stirred for 30 minutes and then quenched with water (5 mL). The organic layer was washed with a saturated $NaHCO_3$ solution until pH 7, then water (3 × 5 mL) and brine (5 mL). The organic layer was dried over $MgSO_4$ and then concentrated under reduced pressure. The crude product was purified by silica column chromatography (Hex/EtOAc: 8/2–75/25 v/v) to yield (**31**) (0.060 g, 71.2%) as white foam. δ_H (500 MHz; $CDCl_3$; Me_4Si) 7.67–7.65 (4H, m, Ph), 7.45–7.35 (7H, m, Ph), 7.32–7.26 (9H, m, Ph), 4.72 (1H, d, J = 11.4 Hz, CH_2–Ph), 4.63 (1H, d, J = 11.4 Hz, CH_2–Ph), 4.59 (2H, s, CH_2–Ph), 4.13–4.12 (2H, m, CH_2–alkyne), 3.97–3.75 (5H, m, H-1, H-3, H-4, H-5), 3.73–3.71 (1H, m, H-6), 3.67–3.64 (1H; m, H-6), 2.39 (1H, t, J = 2.4 Hz, CH≡C), 1.89–1.81 (2H, m, H-2), 1.07 (9H, s, (CH_3)). δ_C (125 MHz; $CDCl_3$; Me_4Si) 138.5, 138.0, 135.6, 135.5, 133.6(2), 129.6, 128.3, 128.1, 127.7, 127.6(2), 80.1, 80.0 (C≡CH), 79.4 (C-3), 75.6 (C-4), 74.7 (CH≡C), 74.3, 72.7 (CH_2–Ph), 61.0 (C-6), 60.2 (C-1), 57.0 (CH_2–alkyne), 33.6 (C-2), 26.9 (CH_3), 19.2 (C–$(CH_3)_3$). HRMS (ES) calculated $[M + H]^+$ = 623.3193; found $[M + H]^+$ = 623.3181.

*3,4-Di-*O*-benzyl-5-*O*-propargyl-6-*O*-trityl-2-deoxy-D-glucitol (32).* To a solution of (**30**) (0.11 g, 0.13 mmol) in THF (10 mL) was added a TBAF (0.15 mL, 0.15 mmol, in THF $C \sim 1$ M). The reaction mixture was stirred overnight at rt then diluted with water (20 mL) and extracted with EtOAc (3 × 10 mL). The organic layer was washed with water (3 × 10 mL), brine (10 mL) and dried over $MgSO_4$. EtOAc was removed under reduced pressure until dry. The crude product was purified by silica column chromatography (Hex/EtOAc/TEA: 80/19/1 v/v/v) to yield (**32**) (0.067 g, 79%) as a white foam. δ_H (500 MHz; $CDCl_3$; Me_4Si) 7.46–7.44 (5H, m, Ph), 7.27–7.19 (18H, m, Ph), 7.10–7.08 (2H, m, Ph), 4.65 (1H, d, J = 11.4 Hz, CH_2–Ph), 4.57–4.49 (3H, m, CH_2–Ph), 4.34 (1H, dd, J = 2.3 Hz, 15.6 Hz, CH_2–alkyne), 4.20 (1H, dd, J = 2.3 Hz, 15.6 Hz CH_2–alkyne), 3.92–3.90 (1H, m, H-5), 3.82–3.77 (2H, m, H3, H-1), 3.63–3.61 (2H, m, H4, H-1), 3.47 (1H, dd, J = 3.1 Hz, 10.5 Hz, H-6), 3.34 (1H, dd, J = 5.5 Hz, 10.5 Hz, H-6), 2.36 (1H, t, J = 2.3 Hz, HC≡C), 1.77–1.74 (2H, m, H-2). δ_C (125 MHz; $CDCl_3$; Me_4Si) 143.8(3), 138.4, 138.3, 128.7, 128.3, 128.1, 128.0, 127.9, 127.8, 127.6(2), 127.4, 127.0, 86.9 (C–$(Ph)_3$), 80.9 (C-5), 80.1 (C≡CH), 78.6 (C-3), 77.7 (C-4), 74.4 (CH≡C), 74.0, 73.0 (CH_2–Ph), 63.2 (C-1), 60.3 (C-6), 57.8 (CH_2–alkyne), 34.2 (C-2). HRMS (ES) calculated $[M + Na]^+$ = 649.2930, found $[M + Na]^+$ = 649.2940.

*3,4-Di-*O*-benzyl-1-*O*-(dibenzylphosphatyl)-5-*O*-propargyl-6-*O*-trityl-2-deoxy-D-glucitol (34).* Using the procedure B, (**32**) (0.067 g, 0.11 mmol) was phosphorylated and purified by column chromatography (Hex/EtOAc/TEA: 9/0.9/1–8/1.9/0.1 v/v/v) to yield (**34**) (0.060 g, 63%) as a colourless oil. δ_H (500 MHz; $CDCl_3$; Me_4Si) 7.42–7.40 (7H, m, Ph), 7.27–7.14 (29H, m, Ph), 4.96 (2H, s, CH_2–OP), 4.95 (2H, m, CH_2–OP), 4.51 (1H, d, J = 11.4 Hz, CH_2–Ph), 4.44 (1H, d, J = 3.4 Hz, CH_2–Ph), 4.41 (1H, d, J = 3.4 Hz, CH_2–Ph), 4.38 (1H, d, J = 11.4 Hz, CH_2–Ph), 4.27 (1H, dd, J = 2.4 Hz, 15.5 Hz, CH_2–alkyne), 4.07 (1H, dd, J = 2.4 Hz, 15.5 Hz, CH_2–alkyne), 4.00 (2H, quad., J = 6.5 Hz, H-1), 3.84 (1H, td, J = 2.6 Hz, 5.9 Hz, H-5), 3.79–3.76 (1H, m, H-3), 3.67 (1H, dd, J = 4.2 Hz, 5.9 Hz, H-4), 3.44 (1H, dd, J = 2.6 Hz, 10.6 Hz, H-6), 3.25 (1H, dd, J = 5.6 Hz, 10.6 Hz, H-6), 2.29 (1H, t, J = 2.4 Hz, CH≡C), 1.83–1.79 (2H, m, H-2). δ_C (125 MHz; $CDCl_3$; Me_4Si) 143.6(3), 138.5, 138.2, 135.6 (d, J = 6.8 Hz), 133.4 (d, J = 5.1 Hz), 128.8 128.7, 128.4, 128.1(2), 128.0, 127.9, 127.8 (2), 127.7, 127.5, 127.4, 127.2, 126.9, 86.7, 80.0 (C≡CH), 78.5 (C-5), 77.7 (C-3), 77.5 (C-4), 74.5 (CH≡C), 73.6, 73.0 (CH_2–Ph), 69.0 (d, J = 5.8 Hz, CH_2–OP), 68.3 (d, J = 5.8 Hz, CH_2–OP), 66.7 (d, J = 5.1 Hz, C1), 63.5 (C6), 57.6 (CH_2–alkyne), 35.4 (d, J = 7.1 Hz, C-2). δ_p (121 MHz; $CDCl_3$; Me_4Si) 0.46 (s, decoupled).

HRMS (ES) calculated $[M + Na]^+$ = 909.3532, found $[M + Na]^+$ = 909.3565.

1-[2,3,5-Tri-O-benzoyl-β-D-ribofuranosyl]-4-methylenoxy-[5-(3S,4R,-5R)-3,4-di-O-benzyl-1-O-(dibenzylphosphatyl)-6-O-trityl-hexanyl]-1H-1,2,3-triazole (37). Using procedure C, alkyne (**32**) (0.23 g, 0.27 mmol) was mixed with azido-2,3,5-tri-*O*-benzoyl-β-D-ribofuranoside (**11**) (0.14 g, 0.30 mmol) in a water–*t*BuOH (5 mL) mixture. A pre-mixed copper sulfate (0.027 g, 0.11 mmol), sodium ascorbate (0.043 g, 0.22 mmol) aqueous solution (5 mL) was added to the previous suspension. After 8 hours, the crude material was purified by silica column chromatography (Hex/EtOAc/TEA: 75/24/1–1/0.9/0.1 v/v/v) to give (**37**) (0.24 g, 71%) as a colourless oil. δ_H (500 MHz; $CDCl_3$; Me_4Si) 8.01–7.94 (8H, m, Ph), 7.75 (1H, s, H triazole), 7.59–7.53 (3H, m, Ph), 7.48–7.33 (20H, m, Ph), 7.38–7.28 (17H, m, Ph), 7.09–7.07 (2H, m, Ph), 6.27 (1H, d, J = 3.1 Hz, H-1′), 6.23 (1H, t, J = 3.1 Hz, H-2′), 6.14 (1H, t, J = 6.0 Hz, H-3′), 5.00–4.95 (1H, m, CH_2–OP), 4.89–4.84 (3H, m, CH_2–OP), 4.75 (1H, dd, J = 3.4 Hz, 12.2 Hz, CH_2–triazole), 4.65–4.43 (7H, m, CH_2–Ph, CH_2–triazole, H-1), 3.86–3.79 (3H, m, H-5′, H-5), 3.73 (1H, t, J = 5.5 Hz, H-4), 3.66–3.63 (1H, m, H-3), 3.54 (1H, dd, J = 3.1 Hz, 10.4 Hz, H-6), 3.38 (1H, dd, J = 5.7 Hz, 10.4 Hz, H-6), 1.81–1.68 (2H, m, H-2). δ_C (125 MHz; $CDCl_3$; Me_4Si) 166.1, 165.1, 165.0 (C═O), 146.2 (C triazole), 143.9(3), 138.5, 138.3, 135.6 (d, J = 6.8 Hz), 133.8, 133.6, 133.3 (d, J = 3.3 Hz) 129.8(2), 129.7, 129.3, 128.7, 128.7, 128.6, 128. 5(2), 128.4, 128.3, 128.1(2), 128.0, 127.9, 127.8(2), 127.6, 127.4, 127.0, 122.1 (CH triazole), 90.2 (C-1′), 87.0 (C–(Ph)3), 81.0 (C-4′), 80.6 (C-4), 80.0 (C-5), 77.6 (C-2′), 75.2 (C-3), 74.0, 73.9 (CH_2–Ph), 71.7 (C-3′), 69.2 (d, J = 1.9 Hz), 69.1 (d, J = 1.9 Hz), 64.3 (CH_2–triazole), 63.8 (C-5′), 63.6 (C-6), 60.0 (C-1), 34.1 (C-2). δ_p (121 MHz; $CDCl_3$; Me_4Si) 0.15 (s, decoupled). HRMS (ES) calculated $[M + H]^+$ = 1374.5092, found $[M + H]^+$ = 1374.5063.

1-[2,3-Di-O-acetyl-5-O-(diphenylphosphatyl)-β-D-ribofuranosyl]-4-methylenoxy-[5-(3S,4R,5R)-3,4-di-O-benzyl-1-O-(dibenzylphosphatyl)-6-O-trityl)-hexanyl]-1H-1,2,3-triazole (38). Using procedure C, (**34**) (0.24 g, 0.27 mmol) was mixed with azido-2,3-di-*O*-acetyl-5-*O*-(diphenylphosphatyl)-β-D-ribofuranoside (**14**) (0.16 g, 0.30 mmol) in a water–*t*BuOH (5 mL) mixture. A pre-mixed copper sulfate (0.028 g, 0.11 mmol), sodium ascorbate (0.044 g, 0.22 mmol) aqueous soltion (5 mL) was added to the previous suspension. After 6 hours, the crude material was purified by silica column chromatography (Hex/EtOAc/TEA: 75/24/1–6/3.9/0.1 v/v/v) to give (**38**) (0.24 g, 62%) as a colourless oil. δ_H (500 MHz; $CDCl_3$; Me_4Si) 7.74 (1H, s, H triazole), 7.44–7.42 (8H, m, Ph), 7.27–7.15 (44H, m, Ph), 7.02–7.01 (2H, m, Ph), 6.01 (1H, d, J = 3.9 Hz, H-1′), 5.76 (1H, dd, J = 3.9 Hz, 5.4 Hz, H-2′), 5.63 (1H, t, J = 5.4 Hz, H-3′), 4.98–4.92 (8H, m, CH_2–OP), 4.83–4.80 (1H, d, J = 12.0 Hz, CH_2–Ph), 4.55–4.51 (3H, t, J = 12.0 Hz, CH_2–Ph), 4.42–4.36 (3H, m, H-4′, CH_2–triazole), 4.19–4.08 (3H, m, H-5′, H-1), 3.83–3.81 (1H, m, H-3), 3.79–3.75 (1H, m H-5), 3.68–3.66 (1H, m, H-4), 3.52 (1H, dd, J = 2.8 Hz, 10.5 Hz, H-6), 3.32 (1H, dd, J = 5.5 Hz, 10.5 Hz, H-6), 2.06 (3H, s, CH_3), 2.03 (3H, s, CH_3), 1.85–1.81 (1H, m, H-2), 1.79–1.74 (1H, m, H-2). δ_C (125 MHz; $CDCl_3$; Me_4Si) 169.2, 168.9 (C═O), 145.9 (C triazole), 143.9(3), 138.4, 138.1, 135.8(2) (d, J = 6.7 Hz), 135.5, 135.4 (d, J = 6.7 Hz), 128.7, 128.4(2), 128.2, 128.1, 127.9(2), 127.8(2), 127.3(2), 126.9, 122.3 (CH triazole), 89.7 (C-1′), 86.9 (C–$(Ph)_3$), 81.2 (d, J = 7.6 Hz, C-4′), 80.1 (C-4), 79.6 (C-5), 75.4 (C-2′), 75.3 (C-3), 73.7, 72.6 (CH_2–Ph), 70.7 (C-3′), 69.5, 69.0 (t, J = 5.2 Hz, CH_2–OP), 66.4 (d, J = 5.3 Hz, C-5′), 64.9 (d, J = 5.9 Hz, C-1), 64.3 (CH_2–triazole), 63.4 (C-6), 30.2 (d, J = 7.1 Hz, C-2), 20.4, 20.3 (CH_3). δ_p (121 MHz; $CDCl_3$; Me_4Si) 0.15 (s, decoupled), −0.01 (s, decoupled). HRMS (ES) calculated $[M + H]^+$ = 1406.5120, found $[M + H]^+$ = 1406.5144.

*1-[2,3,5-Tri-O-benzoyl-β-D-ribofuranosyl]-4-methylenoxy-[5-(3S,4R,-5R)-3,4-di-O-benzyl-6-O-(dibenzylphosphatyl)-1-O-(*tert*-butyl-diphenylsilyl)-hexanyl]-1H-1,2,3-triazole (39).* Using the procedure A, the alkyne precursor (0.31 g, 0.50 mmol) was phosphorylated and the crude passed through a silica column chromatography (Hex/EtOAc: 9/1–8/2 v/v) to give the phosphorylated intermediate (**34**) as a crude colourless oil. Using procedure B, the whole quantity of this crude was mixed with azido-2,3,5-tri-*O*-benzoyl-β-D-ribofuranoside (**11**) (0.27 g, 0.55 mmol) in a water–*t*BuOH mixture (5 mL, 1 : 1 v/v). A premix aqueous solution (5 mL) of copper sulfate (0.050 g, 0.20 mmol) and sodium ascorbate (0.079 g, 0.40 mmol) was added to the suspension. After 6 hours, the crude product was purified by column chromatography (Hex/EtOAc: 75/25 v/v) to give (**39**) (0.30 g, 44% overall) as a colourless oil. δ_H (500 MHz; $CDCl_3$; Me_4Si) 8.02–7.99 (4H, m, Ph), 7.96–7.94 (2H, m, Ph), 7.72 (1H, s, H triazole), 7.65–7.62 (5H, m, Ph), 7.44–7.22 (3H, m, Ph), 7.20–7.10 (31H, m, Ph), 6.25 (1H, dd, J = 2.8 Hz, 5.5 Hz, H-2′), 6.19 (1H, d, J = 5.5 Hz, H-3′), 6.16 (1H, d, J = 2.8 Hz, H-1′), 5.04–5.02 (4H, dd, J = 5.7 Hz 7.9 Hz, CH_2–OP), 4.87–4.84 (1H, m, H-4′), 4.72 (1H, dd, J = 3.95 Hz, 12.1 Hz, CH_2–triazole), 4.68–4.65 (1H, d, J = 12.1 Hz, H-5′), 4.65–4.62 (2H, m, CH_2–triazole, H-5′), 4.55–4.53 (4H, m, CH_2–Ph), 4.44–4.41 (1H, dd, J = 2.1 Hz, 6.0 Hz, H-6), 4.26–4.21 (1H, dd, J = 6.0 Hz, 10.5 Hz, H-6), 3.97–3.94 (1H, m, H-3), 3.87–3.85 (1H, m, H-5), 3.78–3.74 (1H, m, H-1), 3.70–3.65 (2H, m, H-1, H-4), 1.91–1.84 (1H, m, H-2), 1.82–1.76 (1H, m, H-2), 1.05 (9H, s, (CH_3)). δ_C (125 MHz; $CDCl_3$; Me_4Si) 166.0, 165.0, 164.9 (C═O), 145.7 (C triazole), 138.5, 138.0, 135.9 (d, J = 2.1 Hz), 135.8 (d, J = 2.1 Hz), 135.6, 133.8, 133.7, 133.5, 133.2, 133.7(2), 129.8(2), 129.6(2), 129.4, 128.8, 128.7, 128.5(2), 128.4(2), 128.3(2), 127.9, 127.8(2), 127.8(3), 127.6(3), 127.5, 122.7 (CH triazole), 90.2 (C-1′), 80.9 (C-4′), 79.3 (C-4), 79.0 (d, J = 7.0 Hz, C-5), 75.5 (C-3), 75.2 (C-2′), 73.9, 72.6 (CH_2–Ph), 71.8 (C-3′), 69.2 (t, J = 4.9 Hz, CH_2–OP,), 67.4 (d, J = 5.8 Hz, C-6), 64.0 (C-5′), 63.9 (CH_2–triazole), 60.4 (C-1), 33.5 (C-2), 26.9 CH_3), 19.2 (C–$(CH_3)_3$). δ_p (121 MHz; $CDCl_3$; Me_4Si) 0.10 (s, decoupled). HRMS (ES) calculated $[M + H]^+$ = 1370.5175, found $[M + H]^+$ = 1370.5187.

*1-[2,3-Di-O-acetyl-5-O-(diphenylphosphatyl)-β-D-ribofuranosyl]-4-methylenoxy-[5-(3S,4R,5R)-3,4-di-O-benzyl-6-O-(dibenzylphosphatyl)-1-O-(*tert*-butyldiphenylsilyl)-hexanyl]-1H-1,2,3-triazole (36).* Using the procedure A, the alkyne precursor (0.31 g, 0.45 mmol) was phosphorylated and partially purified by column chromatography (Hex/EtOAc: 9/1–8/2 v/v) to give phosphorylated intermediate (**32**) as a crude colourless oil. Using procedure C, the whole quantity of this crude was mixed with

azido-2,3-di-*O*-acetyl-5-*O*-(diphenylphosphatyl)-β-D-ribofuranoside (**14**) (0.28 g, 0.55 mmol) in a water–*t*BuOH mixture (5 mL, 1 : 1 v/v). A pre-mix aqueous solution (5 mL) of copper sulfate (0.049 g, 0.20 mmol) and sodium ascorbate (0.078 g, 0.40 mmol) was added to the suspension. After 8 hours, the crude product was purified by column chromatography (Hex/EtOAc: 75/25 v/v) to give (**36**) (0.27 g, 39% overall) as a colourless oil. δ_H (500 MHz; $CDCl_3$; Me_4Si) 7.68 (1H, s, H triazole), 7.63–7.61 (4H, m, Ph), 7.40–7.20 (36H, m, Ph), 5.93 (1H, d, J = 3.8 Hz, H-1′), 5.75 (1H, t, J = 5.2 Hz, H-2′), 5.63 (1H, t, J = 5,2 Hz, H-3′), 5.00–4.96 (8H, m, CH_2–OP), 4.63–4.47 (6H, m, CH_2–Ph), 4.41–4.38 (2H, m, H-4′, H-5′), 4.23–4.10 (4H, m, H-6, H-5′), 3.93–3.89 (1H, m, H-3), 3.84–3.81 (1H, m, H-5), 3.75–3.67 (1H, m H-1), 3.66–3.62 (1H, m, H-1), 2.07 (3H, s, CH_3), 2.06 (3H, s, CH_3), 1.78–1.71 (2H, m, H-2), 1.02 (9H, s, CH_3). δ_C (125 MHz; $CDCl_3$; Me_4Si) 169.2, 169.1 (C=O), 145.6 (C triazole), 138.5, 138.0, 135.9, 135.8 (d, J = 2.8 Hz), 135.6(2), 135.5, 133.7(2) (d, J = 5.3 Hz), 129.6(2), 128.5(2), 128.4(2), 128.3(2), 128.2(2), 128.0(2), 127.9(2), 127.8, 127.6(2), 127.5(2), 122.6 (CH triazole), 89.7 (C-1′), 81.3 (d, J = 7.6 Hz, C-4′), 79.4 (C-4), 79.1 (d, J = 7.1 Hz, C-5), 75.5 (C-3), 75.2 (C-2′), 73.2, 72.6 (CH_2–Ph), 70.7 (C-3′), 69.5 (t, J = 4.7 Hz, CH_2–OP), 69.2 (t, J = 4.7 Hz, CH_2–OP,), 67.5 (d, J = 5.8 Hz, C-5,), 66.4 (d, J = 5.6 Hz, C-6), 63.9 (CH_2–triazole), 60.4 (C-1), 33.5 (C-2), 26.9 (CH_3), 20.4, 20.3 (CH_3), 19.1 (C–$(CH_3)_3$). δ_p (121 MHz; $CDCl_3$; Me_4Si) 0.12 (s, decoupled), 0.02 (s, decoupled). HRMS (ES) calculated $[M + H]^+$ = 1402.5284, found $[M + H]^+$ = 1402.5277.

*1-[5-*O*-(Dibenzylphosphatyl)-β-D-ribofuranosyl]-4-methylenoxy-[5-(3*S*,4*R*,5*R*)-3,4-di-*O*-benzyl-1-*O*-(*tert*-butyldiphenylsilyl)-6-O-trityl-hexanyl]-1*H*-1,2,3-triazole (**35**).* Using procedure B, (**30**) (0.060 g, 0.069 mmol) was mixed with azido-3,5-di-*O*-acetyl-5-*O*-(dibenzylphosphatyl)-β-D-ribofuranoside (**14**) (0.040 g, 0.076 mmol) in a water–*t*BuOH (1 mL) mixture. A pre-mixed copper sulfate (0.007 g, 0.030 mmol), sodium ascorbate (0.011 mg, 0.060 mmol) aqueous solution (1 mL) was added to the suspension. After 8 hours, the crude product was dissolved in MeOH (3 mL) and MeONa (0.1 mL 25% wt in MeOH) was added dropwise and was stirred for 20 minutes, then concentrated. The crude material was purified by column chromatography (Hex/EtOAc/TEA: 8/1.9/0.1–0/0.9/0.1 v/v/v) to give (**35**) (0.025 g, 28% overall) as a colourless oil. δ_H (500 MHz; $CDCl_3$; Me_4Si) 7.65–7.60 (4H, m, Ph), 7.44–7.21 (44H, m, Ph), 7.00–6.97 (2H, m, Ph), 5.87 (1H, d, J = 1.8 Hz, H-1′), 5.09–5.00 (4H, m, CH_2–OP), 4.81 (1H, d, J = 11.5 Hz, CH_2–Ph), 4.79 (1H, d, J = 11.8 Hz, CH_2–triazole), 4.65–4.62 (3H, m, CH_2–Ph), 4.61 (1H, d, J = 11.8 Hz, CH_2–triazole), 4.53 (2H, m, H-5′), 4.31–4.27 (1H, m, H-4′), 4.20–4.18 (1H, m, H-2′), 4.05–4.01 (1H, m, H-5), 4.01–3.96 (5H, m, H-1, H-3, H4, H-3′), 3.55 (1H, dd, J = 2.4 Hz, 10.5 Hz, H-6), 3.30 (1H, dd, J = 5.6 Hz, 10.5 Hz, H-6), 1.77–1.72 (2H, m, H-2), 1.08 (9H, s, (CH_3). δ_C (125 MHz; $CDCl_3$; Me_4Si) 145.9 (C triazole), 143.4(3), 137.6, 136.7(2), 135.5, 135.3 (d, J = 7.0 Hz), 133.2 (d, J = 3.3 Hz), 133.0, 132.9, 130.1, 129.4, 128.5, 128.3, 128.2, 128.1, 127.9, 127.8(2), 127.7(2), 127.6, 127.5, 127.4, 122.7 (CH triazole), 90.0 (C-1′), 86.6 (C–$(Ph)_3$), 81.0 (d, J = 7.4 Hz, C-4′), 80.2 (C-4), 78.5 (C-2), 77.6 (C-5), 77.1 (C-3), 73.7, 73.0 (CH_2–Ph), 72.4 (C-2′), 69.6(2) (d, J = 3.7 Hz, CH_2–OP), 68.7 (C-3′), 66.3 (d, J = 6.0 Hz, C-5′), 63.9 (CH_2–triazole), 63.5 (C-6), 62.9 (C-1), 33.2 (C-2), 26.1 (CH_3), 19.0 (C–$(CH_3)_3$). δ_p (121 MHz; $CDCl_3$; Me_4Si) −0.13 (s, decoupled). HRMS (ES) calculated $[M + Na]^+$ = 1322. 5303, found $[M + Na]^+$ = 1322.5309.

*1-(β-D-Ribofuranosyl)-4-methylenoxy-[5-(3*S*,4*R*,5*R*)-1-O-(phosphatyl)-hexan-3,4,6-triolyl]-1*H*-1,2,3-triazole (**41**).* Using procedure D, compound (**39**) (160 mg, 0.098 mmol) was deprotected and purified to give compound (**41**) (39 mg, 87% overall) as a white gum. δ_H (500 MHz; D_2O; Me_4Si) 8.11 (1H, s, H triazole), 6.00 (1H, d, J = 3.9 Hz, H-1′), 4.27 (1H, t, J = 5.2 Hz, H-2′), 4.07 (1H, q, J = 5.2 Hz, H-3′), 3.87–3.70 (5H, m, H-4, CH_2–triazole, H-5′, H-5, H-4′), 3.67 (1H, d, J = 3.2 Hz, CH_2–triazole), 3.60–3.58 (2H, m, H-1), 3.56–3.49 (2H, m, H-6, H-3), 3.41 (1H, dd, J = 2.0 Hz, 7.7 Hz, H-6), 1.73–1.64 (2H, m, H-2). δ_C (125 MHz; D_2O; Me_4Si) 144.5 (C triazole), 124.3 (CH triazole), 92.4 (C-1′), 85.5 (C-4′), 78.3 (C-5), 75.1 (C-4), 73.5 (C-3), 71.7 (C-2′), 66.8 (C-3), 63.6 (d, J = 4.9 Hz, C-1), 62.4 (C-5′), 61.5 (CH_2–triazole), 60.0 (C-6), 34.1 (d, J = 6.9 Hz, C-2). δ_p (121 MHz; D_2O; Me_4Si) 1.16 (br, decoupled). HRMS (ES) calculated $[M - H]^-$ = 458.1176, found $[M - H]^-$ = 458.1164.

*1-[(5-O-Phosphatyl)-β-D-ribofuranosyl]-4-methylenoxy-[5-(3*S*,4*R*,5*R*)-1-O-(phosphatyl)-hexane-3,4,6-triolyl]-1*H*-1,2,3-triazole (**40**).* Using procedure D, compound (**38**) (52 mg, 0.037 mmol) was deprotected to give (**40**) (18 mg, 90% overall) as a white gum. δ_H (500 MHz; D_2O; Me_4Si) 8.21 (1H, s, H triazole), 6.10 (1H, d, J = 4.6 Hz, H-1′), 4.58 (1H, q, J = 4.6 Hz, H-2′), 4.38 (1H, t, J = 4.6 Hz, H-3′), 4.30–4.27 (1H, m, H-4′), 4.06–3.93 (5H, m, H-4, CH_2–triazole, H-5′), 3.86–3.81 (2H, m, H-5, H-3), 3.67–3.55 (3H, m, H-1, H-6), 3.49–3.46 (1H, m, H-6), 1.80–1.71 (2H, m, H-2). δ_C (125 MHz; D_2O; Me_4Si) 143.1 (C triazole), 123.7 (CH triazole), 92.6 (C-1′), 84.4 (d, J = 8.5 Hz, C-4′), 79.5 (C-5), 75.4 (C-4), 71.8 (C-3), 70.6 (C-2′), 66.8 (C-3′), 65.0 (d, J = 4.6 Hz, C-1), 63.4 (d, J = 5.0 Hz, C-5′), 62.5 (CH_2–triazole), 60.1 (C-6), 34.1 (d, J = 6.8 Hz, C-2). δ_p (121 MHz; D_2O; Me_4Si) 4.20 (s, decoupled), 3.12 (s, decoupled). HRMS (ES) calculated $[M - H]^-$ = 538.0839, found $[M - H]^-$ = 538.0809.

*1-(β-D-Ribofuranosyl)-4-methylenoxy-[5-(3*S*,4*R*,5*R*)-6-O-(phosphatyl)-hexan-1,2,3,4-tetraolyl]-1*H*-1,2,3-triazole (**44**).* Using procedure D, compound (**37**) (31 mg, 0.023 mmol) was deprotected and purified to give compound (**44**) (7 mg, 68% overall) as a pure white gum. δ_H (500 MHz; D_2O; Me_4Si) 8.13 (1H, s, H triazole), 5.88 (1H, dd, J = 1.8 Hz, 3.8 Hz, H-1′), 4.53 (1H, td, J = 1.8 Hz, 5.8 Hz, H-2′), 4.26 (1H, t, J = 51.8 Hz, H-3′), 4.67–4.62 (2H, m, H-3, H-4), 4.10–4.06 (2H, m, CH_2–triazole), 3.91–3.87 (1H, m, H-5), 3.79–3.77 (1H, m, H-4′), 3.70–3.64 (2H, m, H-5′, H-6), 3.58–3.53 (3H, m, H-1, H-6, H-5′), 3.41–3.39 (1H, m, H-1), 1.70–1.63 (1H, m, H-2), 1.56–1.53 (1H, m, H-2). δ_C (125 MHz; D_2O; Me_4Si) 143.2 (C triazole), 122.6 (CH triazole), 90.1 (C-1′), 83.3 (C-4′), 78.3 (d, J = 7.2 Hz, C-5), 75.4 (C-4), 71.7 (C-3), 70.7 (C-2′), 67.2 (C-3′), 64.5 (d, J = 3.5 Hz, C-6), 63.0 (CH_2–triazole), 60.4 (C-5′), 58.1 (C-1), 35.6 (C-2). δ_p (121 MHz; D_2O; Me_4Si) 1.78 (s, decoupled). HRMS (ES) calculated $[M + Na]^+$ = 482.1152, found $[M + Na]^+$ = 482.1114.

*1-[(5-O-Phosphatyl)-β-D-ribofuranosyl]-4-methylenoxy-[5-(3*S*,4*R*,5*R*)-6-O-(phosphatyl)-hexan-1,3,4-triolyl]-1*H*-1,2,3-triazole (**43**).* Using procedure D, compound (**36**) (0.11 g, 0.077 mmol) was

deprotected and purified to give (**43**) (0.032 g, 76% overall) as a white gum. δ_H (500 MHz; D_2O; Me_4Si) 8.19 (1H, s, H triazole), 6.00 (1H, d, J = 4.6 Hz, H-1′), 4.50 (1H, q, J = 4.9 Hz, H-2′), 4.31 (1H, t, J = 4.6 Hz, H-3′), 4.25–4.23 (1H, m, H-4′), 4.13–4.06 (3H, m, CH_2–triazole, H-4), 3.98–3.87 (2H, m, H-5′), 3.81–3.76 (1H, m, H-5), 3.68–3.62 (1H, m, H-3), 3.57–3.53 (2H, m, H-1), 3.43–3.37 (1H, m, H-6), 3.05–2.99 (1H, m, H-6), 1.22–1.04 (2H, m, H-2). δ_C (125 MHz; D_2O; Me_4Si) 142.1 (C triazole), 121.9 (CH triazole), 91.3 (C-1′), 84.6 (d, J = 7.0 Hz, C-4′), 78.0 (d, J = 7.4 Hz, C-5), 75.3 (C-4), 71.5 (C-3), 70.8 (C-2′), 66.9 (C-3′), 65.0 (d, J = 3.0 Hz, C-6), 64.3 (CH_2–triazole), 61.7 (d, J = 4.1 Hz, C-5′), 59.0 (C-1), 35.7 (C-2). δ_p (121 MHz; D_2O; Me_4Si) 1.36 (s, decoupled), 1.30 (s, decoupled). HRMS (ES) calculated $[M - H]^-$ = 538.0839, found $[M - H]^-$ = 538.0831.

*1-[(5-O-Phosphatyl)-β-D-ribofuranosyl]-4-methylenoxy-[5-(3S,4R,-5R)-hexan-1,3,4,6-tetraolyl]-1H-1,2,3-triazole (**42**).* Using procedure D, compound (**35**) (170 mg, 0.13 mmol) was deprotected and purified to give (**42**) (45 mg, 75% overall) as a pure white gum. δ_H (500 MHz; D_2O; Me_4Si) 8.19 (1H, s, H triazole), 6.01 (1H, d, J = 4.7 Hz, H-1′), 4.49 (1H, t, J = 4.8 Hz, H-2′), 4.30 (1H, t, J = 4.8 Hz, H-3′), 4.22–4.19 (1H, m, H-4′), 3.95–3.71 (6H, m, H-5, H-5′, H-4, CH_2–triazole), 3.58–3.47 (4H, m, H-6, H-3, H-1), 3.39–3.35 (1H, m, H-6), 1.67–1.60 (1H, m, H-2), 1.57–1.51 (1H, m, H-2). δ_C (125 MHz; D_2O; Me_4Si) 142.9 (C triazole), 122.6 (CH triazole), 92.7 (C-1′), 84.7 (d, J = 8.2 Hz, C-4′), 79.5 (C-5), 75.6 (C-4), 72.0 (C-3), 70.9 (C-2′), 67.3 (C-3′), 64.9 (d, J = 4.7 Hz, C-5′), 62.7 (C-6), 60.2 (CH_2–triazole), 60.0 (C-1), 35.8 (C-2). δ_p (121 MHz; D_2O; Me_4Si) 1.83 (s, decoupled). HRMS (ES) calculated $[M + Na]^+$ = 482.1152, found $[M + Na]^+$ = 482.1143.

In vitro assay of isolated yeast mIPS enzyme

Purification of recombinant yeast mIPS. *Saccharomyces cerevisiae* BY4741 *ino1*Δ mutant bearing the pRD*INO1* construct was used to express the recombinant 6xHis-tagged mIPS. Cells were grown at 30 °C in synthetic minimal medium lacking uracil. Galactose (2%) was used to induce over-expression of the recombinant protein. Cell extracts were prepared by disrupting the cells with glass beads. mIPS was purified by affinity chromatography using ProBond nickel-chelating resin to bind the protein. The resin was washed twice with 20 mM then 60 mM imidazole in Tris buffer (50 mM Tris-Cl (pH 7.4) and 300 mM NaCl). The protein was eluted with 250 mM imidazole in Tris buffer, dialyzed, concentrated, and resuspended in (50 mM Tris-Cl, 50 mM NaCl, 10 mM DTT). Protein concentration was determined by the Bradford method using bovine serum albumin as the standard.

MIPS enzyme assay. Purified mIPS enzyme activity was determined by the rapid colorimetric method of Barnett *et al.*[21] with minor modifications. Purified protein was suspended in the reaction buffer (100 mM Tris acetate (pH 8.0), 0.8 mM NAD, 2 mM DTT, 14 mM NH_4Cl) containing the inhibitor present in various concentrations (0, 100 μM, and 200 μM). 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. The supernatant (200 μL) was incubated with 200 μL of 0.2 M $NaIO_4$ 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 having been released by $NaIO_4$ treatment to control for phosphatase activity or for any phosphates that may have been released from the MAs. This value was subtracted from the experimental sample to obtain mIPS activity. All results were normalised to 100% mIPS activity. Data represents the average of three independent experiments for the 100 μM set, and two independent experiments for the 200 μM set.

Docking experimental methods

Model preparation. It is essential to prepare the crystal structures prior to ligand docking. Crystal structures of 1jki and 1rm0 were prepared using the Protein Preparation Wizard[23] in the Schrödinger Suite 2009. Default parameters were employed for the initial preparation step followed by optimisation of the hydrogen-bond network and a subsequent minimisation using the OPLS-AA 2001 force field. Crystal ligands (1jki: NAD and 2-deoxy-glucitol-6-phosphate, 1rm0: NAD and 2-deoxy-D-glucitol 6-(*E*)-vinylhomophosphonate) were finally omitted from the complex.

Ligand building. All compounds were built and geometries 'cleaned' using the Build panel incorporated in the Maestro[24] graphic interface of the Schrödinger Suite 2009.

Ligand docking. Prior to ligand docking the Glide[25] software requires generation of grids which describe the character of the intended target site. In 1jki the grid box was centred on residues N77, T247, G295, S296, Q325, D356, K369 and K412 and the box dimensions were 32 Å × 32 Å × 32 Å. In the ligand docking step, compounds (**21**), (**22**), (**23**), (**24**), (**40**), (**41**), (**42**), (**43**) and (**44**), were docked into the generated grids. The midpoint of the compounds to be docked was enclosed in a box of dimensions 12 Å × 12 Å × 12 Å, which incorporated the region around the NAD and G6P binding site. The van der Waal radius for ligand atoms was scaled to 0.8. In 1rm0 the grid box was defined by residues N77, A245, S323, G324, Q325, T326, D356, K369, K412 and D438. The remaining steps for 1rm0 ligand docking are the same as those described for 1jki.

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