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A type 2 Ferrier rearrangement-based synthesis of D-*myo*-inositol 1,4,5-trisphosphate

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Dedicated to Professor George Fleet on the occasion of his 65th birthday

ABSTRACT

The synthesis of D-*myo*-inositol 1,4,5-trisphosphate (InsP_3) from methyl α-D-glucopyranose, via a type 2 Ferrier rearrangement is reported. A key intermediate in this synthesis possesses orthogonal protecting groups at the 1-, 4- and 5-position, making it a versatile starting point for the synthesis of unnatural InsP_3 derivatives. Biological evaluation of the synthetic InsP_3 demonstrates that this compound evokes selective Ca^{2+} release via activation of InsP_3 receptors.

1. Introduction

D-*myo*-Inositol 1,4,5-trisphosphate **15** (InsP_3) is a ubiquitous intracellular Ca^{2+}-releasing second messenger that mediates a wide range of cellular functions.[1] InsP_3 is produced within cells in response to GPCR activation by extracellular stimuli and goes on to activate its own intracellular receptors (InsP_3Rs). Activation of InsP_3Rs leads to a rise in intracellular Ca^{2+} concentration that mediates fundamental processes such as fertilisation, behaviour, learning and memory. It is now clear that the InsP_3 signalling cascade resides downstream of most important endogenous chemical transmitters including acetylcholine, adrenaline, dopamine, glutamate and 5-HT, in addition to some tyrosine kinase receptors. InsP_3 is therefore involved in the biological function and hence in dysfunction of these transmitters and the processes that they mediate.[1]

The biochemical importance of InsP_3 has prompted many syntheses of both the naturally occurring molecule and numerous unnatural derivatives. Many of these compounds have proved invaluable tools in the study of the InsP_3Rs.[2,3] Especially useful compounds include the metabolically stable phosphorothioates,[2,3] the adenophostins,[4–11] photoactivated InsP_3 derivatives[12,13] and membrane permeant InsP_3 derivatives.[12,14,15] Despite the numerous InsP_3 derivatives that have been reported, the synthesis of this class of compounds remains challenging. We are interested in the development of selective probes for Ca^{2+} signalling, and in particular InsP_3Rs.[14,16] Therefore, the development of expeditious and versatile synthetic routes, which allow the generation of a variety of InsP_3 derivatives from a common intermediate, is highly desirable. In order to achieve this goal, it is necessary to synthesise inositol derivatives that have orthogonal protecting groups attached to at least three of their oxygen atoms.

As *myo*-inositol is *meso,* syntheses that begin from this cheap and readily available starting material have to overcome the issue of enantioselectivity at some point in the route. With the notable exception of work reported by Miller and co-workers,[17–25] syntheses to single enantiomers of inositol derivatives have largely depended on the resolution of diastereomers.[2,3] One alternative to this approach is the use of chiral pool materials to carry stereochemistry through the synthesis. The most useful chiral pool material for the synthesis of *myo*-inositol derivatives is glucose. The type 2 Ferrier rearrangement[26,27] is an elegant method for the conversion of glucopyranosides into inositol derivatives and there are a number of examples of inositol polyphosphate and phosphatidylinositol polyphosphate derivatives being synthesised using this approach.[28–43] Of these syntheses, only two have focussed on the synthesis of InsP_3,[28,29] and two have focussed on the synthesis of unnatural InsP_3 derivatives.[35,39] However, in all of the above syntheses of InsP_3, the protecting groups employed do not provide suitable orthogonality for the synthesis of the unnatural InsP_3 derivatives in which we are interested. Herein we report a synthetic route, based on the type 2 Ferrier rearrangement, which encompasses orthogonal protecting groups at the 1-, 4- and 5-position and will potentially enable selective elaboration of the 1-,2-,4-,5- and 6-positions of the inositol ring. To exemplify our strategy, we have synthesised InsP_3 and demonstrated that this material releases Ca^{2+} in a permeabilised cell $^{45}Ca^{2+}$ flux assay, in a manner that is consistent with selective activation of InsP_3Rs.

2. Results and discussion

The synthesis of InsP_3 **15** commenced from methyl α-D-glucopyranose **1** (Scheme 1), which was selectively protected at the

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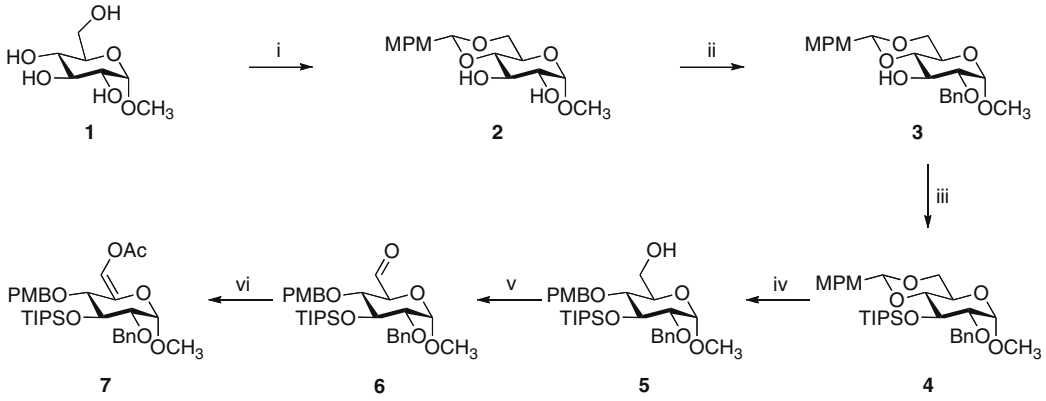
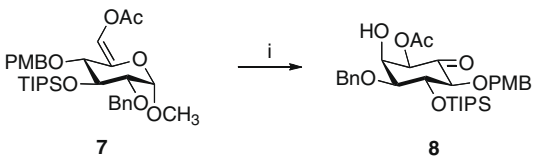


Scheme 1. The synthesis of the Ferrier rearrangement substrate **7**. Reagents and conditions: (i) 4-OMe-PhCH(OMe)$_2$, amberlyst-15, DMF, 200 mbar, 80 °C, 58%; (ii) $^{n}Bu_2SnO$, TBABr, BnBr, MeCN, reflux, 54%; (iii) Et_3N, TIPSOTf, CH_2Cl_2, rt, 92%; (iv) DIBAL-H, CH_2Cl_2, rt, 88%; (v) Dess–Martin Periodinane, CH_2Cl_2, rt, 82%; (vi) K_2CO_3, Ac_2O, DMAP, MeCN, reflux, 65%.

4- and 6-position to give the anisylidene acetal **2**. Treatment of **2** with di-*n*-butyl tin oxide and benzyl bromide in the presence of tetrabutylammonium bromide and 3 Å molecular sieves effected benzylation, with 2:1 selectivity in favour of the desired 2-*O*-benzyl compound **3** over the unwanted regioisomer, and a 54% isolated yield of the desired compound **3**. TIPS protection of the 3-position hydroxyl group was achieved by treatment of **3** with TIPS triflate in the presence of triethylamine, affording **4** in excellent yield. DIBAL-H mediated cleavage of the anisylidene acetal **4** proved to be regioselective, giving an excellent yield of the desired compound, with the PMB group attached to the 4-position of **5**. High yields were obtained in this step only when the quench and work-up procedures were conducted at lower temperature. The 6-position hydroxyl group was oxidised to the corresponding aldehyde **6** using Dess–Martin periodinane. This compound **6** was stable and subjecting it to silica gel column chromatography was found to produce better results in the formation of the enol acetate and subsequent Ferrier rearrangement. The aldehyde **6** was converted to the enol acetate by treatment with potassium carbonate and acetic anhydride, affording the substrate for the Ferrier rearrangement **7**.

The two main metals that have been used to promote the type 2 Ferrier rearrangement of glucose derivatives, in order to give inosose products, are palladium and mercury. Given that palladium is less toxic than mercury and has been employed in substoichiometric quantities, we first investigated the use of palladium to effect the desired rearrangement. However, when **7** was treated with palladium(II) chloride a large number of inseparable isomeric products were observed. This result is not in accordance with the data reported by Takahashi et al.;[28,29] however, we attribute the differences observed to the influence of the TIPS group, which is present in our material but not in that of Takahashi. Gratifyingly, on treatment with mercury(II) acetate, the enol acetate **7** was observed to undergo the type 2 Ferrier rearrangement to afford mainly the desired product **8**, albeit in a low yield (Scheme 2).



Scheme 2. The type 2 Ferrier rearrangement to afford the inosose **8**. Reagents and conditions: (i) (a) $Hg(OAc)_2$, acetone/H_2O (3:2); (b) $NaCl_{(aq)}$, 35%.

Small amounts of isomeric products were also observed, but not fully characterised.

The relative stereochemistry of the inosose ring was confirmed by obtaining an X-ray crystal structure of **8** (Fig. 1). It can be seen that the 2-position hydroxyl group occupies the axial position, which is required for the *myo*-configuration of the inositol ring to be obtained, following reduction of the 6-position ketone.

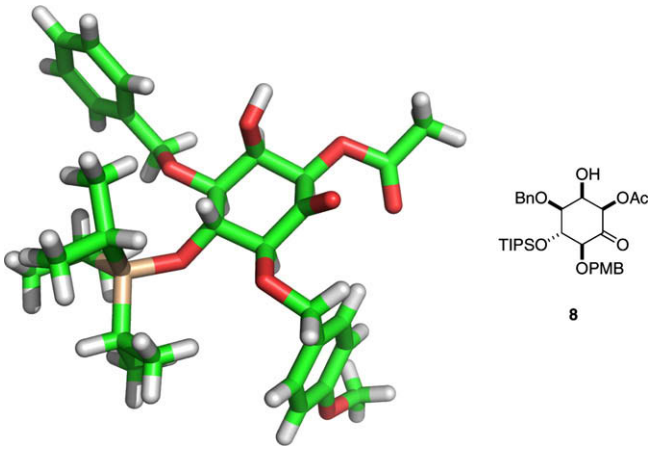
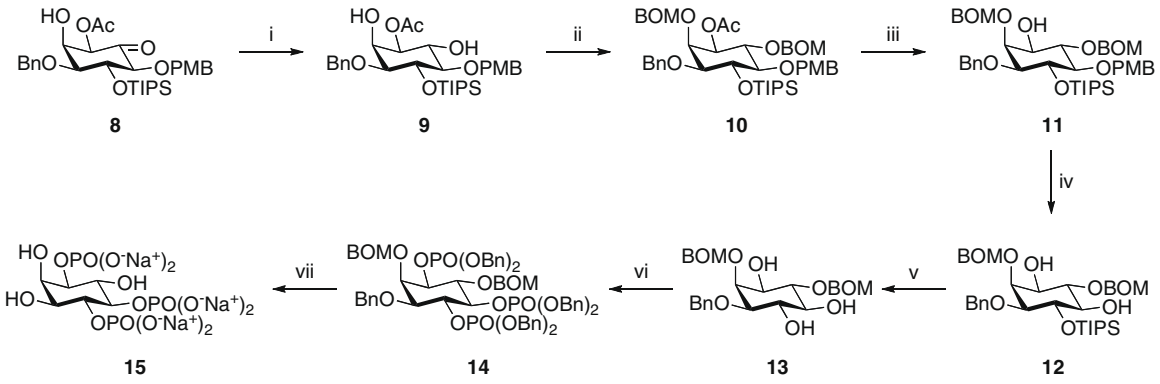


Figure 1. A stick representation of the X-ray crystal structure of compound **8**. The relative stereochemistry of the 1–5 position oxygen atoms can be seen, with the 2-position hydroxyl group sitting in an axial position. Key: carbon, green; oxygen, red; silicon, tan.

The reduction of the 6-position ketone **8** to the inositol **9** was achieved by treatment with tetramethylammonium triacetoxy borohydride (Scheme 3). The stereoselectivity in this reduction is likely as a result of the axial 2-position hydroxyl group co-ordinating to the reducing agent, leading to hydride delivery from the top face and therefore the equatorial hydroxyl group at the 6-positon being obtained.[44] The protection of the 2- and 6-position hydroxyl groups of **9** was required, and benzyl ethers would be the obvious choice of protecting group. It was known from previous work that use of standard conditions, employing sodium hydride and benzyl bromide, would lead to acetate group migration. Hence, the use of benzyl trichloroacetimidate in the presence of a Brønsted or Lewis acid was investigated. Use of trifluoromethanesulfonic acid caused degradation of the starting material **9**, whereas triphenylcarbenium tetrafluoroborate failed to effect the desired reaction. Use of scandium triflate also failed to catalyse benzyl protection, in a

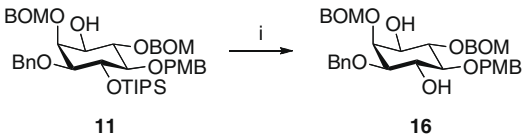


Scheme 3. The synthesis of InsP_3 **15**. Reagents and conditions: (i) $Me_4NBH(OAc)_3$, AcOH, MeCN, 89%; (ii) BOMCl, Hünig's base, 85 °C, 82%; (iii) LiOH, MeOH, THF, 97%; (iv) DDQ, CH_2Cl_2/H_2O (18:1), rt, 95%; (v) TBAF in THF, CH_2Cl_2, rt, 90%; (vi) (a) $(BnO)_2PN(^iPr)_2$, 1*H*-tetrazole (0.43 M in MeCN), CH_2Cl_2, rt; (b) 3-chloroperbenzoic acid, −78 °C→rt, 93%; (vii) H_2, *t*-BuOH, H_2O, Pd black, $NaHCO_3$, 95%.

model system. The use of stronger Lewis acids was rejected, as they were likely to degrade the inositol starting material **9**. We then turned our investigation to the use of Dudley's benzylation conditions,[45] which involve the use of 2-benzyloxy-1-methylpyridinium triflate in the presence of MgO, to transfer the benzyl group to the inositol ring. However, we only ever detected trace amounts of the desired compound. These results are in line with reports from Miller,[18] who also found Dudley's conditions to be ineffective in the benzyl protection of inositols. These results suggest that the inositol hydroxyl groups are too hindered to be benzylated effectively with this reagent.

Given the problems encountered when using benzyl groups, the BOM group was considered as an alternative protecting group. Optimised conditions for BOM protection were found to involve premixing the BOMCl and Hünig's base and then heating the reactants in Hünig's base, affording an 82% yield of the desired material **10**. Deprotection of the acetate **10** afforded the alcohol **11**, which was then treated with DDQ to effect removal of the PMB group, furnishing the diol **12**. Subsequent deprotection of the TIPS group afforded the known triol **13**[28] in good yield. Phosphitylation of the triol **13**, followed by *m*CPBA oxidation, afforded the perbenzylated phosphate **14**. Subsequent palladium black-catalysed hydrogenolysis, in the presence of $NaHCO_3$, afforded the desired InsP_3 **15** as its presumed hexakis sodium salt.

The above order of protecting group removal (Scheme 3) lends itself to the synthesis of 4-position-modified InsP_3 derivatives. As after phosphitylation of the 1- and 5-position hydroxyl groups of **12**, the TIPS group could potentially be removed to leave the 4-position hydroxyl group free and ready for elaboration. With a view to the future synthesis of 5-position-modified InsP_3 derivatives, we investigated an alternative order of protecting group removal (Scheme 4). It was demonstrated that the TIPS group of compound **11** could be removed in the presence of the PMB group, using TBAF in THF. This reaction afforded the 1,4-diol **16**, which could potentially be phosphitylated to afford the fully protected precursor. Removal of the PMB group would leave the 5-position hydroxyl group free and ready for elaboration. Therefore, the above synthesis provides a viable route not only to InsP_3 **15**, but also to two key intermediates in the synthesis of unnatural InsP_3 derivatives **12** and **16**.



Scheme 4. Selective deprotection of the acetate and TIPS of compound **11** to give the intermediate **16**. Reagents and conditions: (i) TBAF in THF, THF, rt, 89%.

In order to confirm that our synthetic InsP_3 exhibited the expected biological action, we investigated its InsP_3-mediated Ca^{2+} mobilising properties in unidirectional Ca^{2+} fluxes from the endoplasmic reticulum (ER) Ca^{2+} stores, in permeabilised Lvec cells. Lvec cells are a stable fibroblast cell line mainly containing type 3 and type 1 InsP_3Rs. Challenging the cells with an InsP_3R agonist leads to a transient increase in the fractional Ca^{2+} loss. Figure 2A shows the data from a typical experiment in permeabilised Lvec cells. The addition of our compound to the efflux medium induced a dose-dependent Ca^{2+} release from the non-mitochondrial intracellular Ca^{2+} stores, a similar response was seen on addition of commercial InsP_3. The Ca^{2+} ionophore A23187[46,47] is used to estimate the maximal releasable Ca^{2+}. The release provoked by our compound or commercially available InsP_3 is normalised to the maximal releasable Ca^{2+} in order to obtain dose–response curves (Fig. 2B). The EC_{50} values of the dose–response curves for synthetic InsP_3 and commercial InsP_3 in Lvec cells show that our compound has a similar potency in stimulating Ca^{2+} release to commercial InsP_3. Similar findings were observed in L15 cells, a stable cell line heterologously overexpressing InsP_3R1 (EC_{50} of our compound = 0.64 mM versus EC_{50} of commercial InsP_3 = 0.91 mM) (data not shown).

In a second investigation, using a unidirectional semi-high throughput Ca^{2+}-flux assay in permeabilised DT40 and triple-InsP_3R knockout cells (TKO), we have examined whether the Ca^{2+}-mobilising properties of the synthetic InsP_3 were dependent on the presence of the InsP_3Rs (Fig. 3). TKO cells are genetically modified DT40 cells, in which all three InsP_3R isoforms have been genomically deleted. In this experiment, the intracellular Ca^{2+} stores of permeabilised DT40 and TKO cells were loaded with the low-affinity Ca^{2+} dye, MagFluo4. Ca^{2+} was loaded in the non-mitochondrial Ca^{2+} stores to steady-state levels using ATP. After reaching the plateau phase, thapsigargin (1 μM) was added to block the SERCA pumps and monitor the unidirectional Ca^{2+} leak from the non-mitochondrial Ca^{2+} stores. Then, 3 μM of either synthetic or commercial InsP_3 was given. Clearly, the synthetic InsP_3 and the commercial InsP_3 provoked a profound Ca^{2+} release from the ER Ca^{2+} stores in permeabilised DT40 cells, but not in TKO cells. This indicates that the synthetic InsP_3 exclusively provokes Ca^{2+} release in an InsP_3R-dependent manner.

3. Conclusion

In conclusion, we have reported a robust synthesis of InsP_3, confirmed the structure of a key intermediate **8** by X-ray

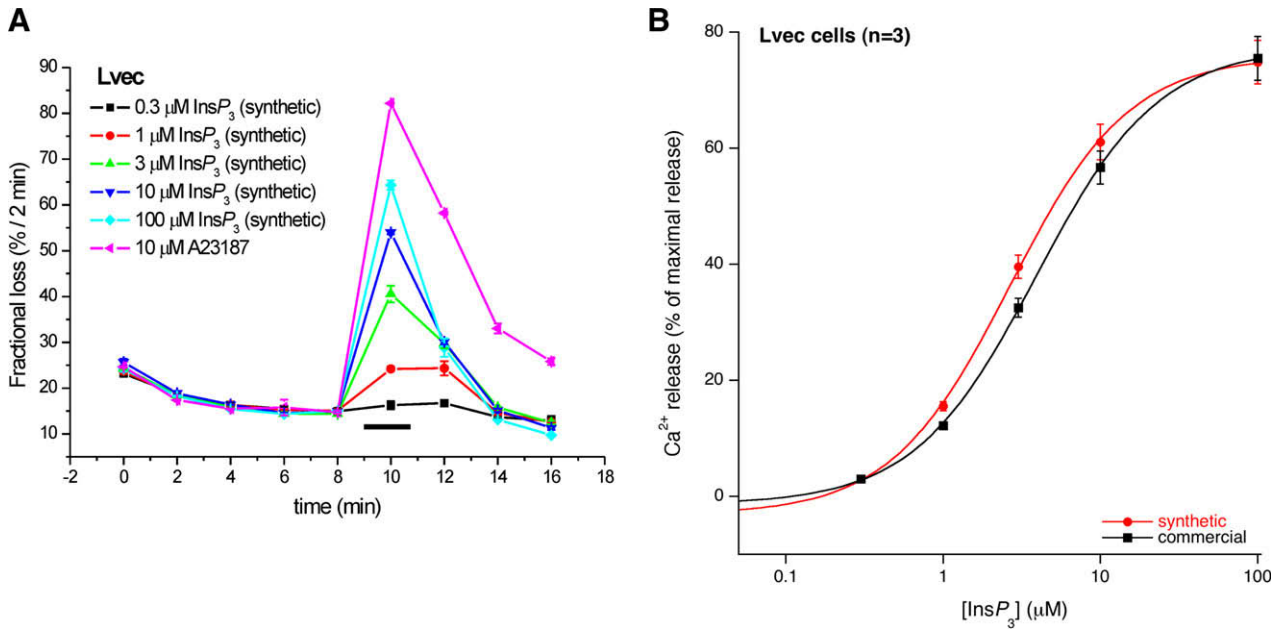


Figure 2. (A) The synthetic InsP_3 mobilises Ca^{2+} from the non-mitochondrial Ca^{2+} stores in permeabilised Lvec cells. A typical experiment showing the Ca^{2+}-mobilising properties of synthetic InsP_3 in Lvec cells. A23187 shows the maximal releasable Ca^{2+}. Data are plotted as fractional loss (%/2 min; the amount of $^{45}Ca^{2+}$ leaving the stores in 2 min divided by the total store Ca^{2+} content at that time) as a function of time. The horizontal bar indicates the addition of InsP_3 or A23187. Results represent the means ± SD and are obtained from duplicate values. (B) The synthetic InsP_3 mobilises Ca^{2+} in permeabilised Lvec cells. Dose response curves showing the Ca^{2+} release provoked by synthetic and commercial InsP_3 in permeabilised Lvec cells and normalised to the A23187-releasable Ca^{2+}. Results represent the means ± SEM of at least three independent experiments each performed in duplicate.

crystallographic analysis and demonstrated that the final compound **15** releases Ca^{2+} in a $^{45}Ca^{2+}$ assay, in a manner consistent with selective InsP_3R activation. More importantly, we have shown that compound **11** may be a key intermediate in the synthesis of both 4- and 5-position-modified InsP_3 derivatives, as sequential deprotection of the PMB group or the TIPS group, in either order, is possible. Given that it is possible to add an orthogonal protecting group at the 2-position prior to reduction and then a further protecting group at the 6-position, this route should prove a versatile means of synthesising a large number of unnatural InsP_3 derivatives, which may prove useful tools for the study of intracellular Ca^{2+} signalling.

4. Experimental

4.1. General experimental details

^{1}H NMR spectra were recorded at 300 MHz, 400 MHz or 500 MHz, on Bruker Avance spectrometers, using deuterochloroform (or other indicated solvent) as reference and as internal deuterium lock. The chemical shift data for each signal are given as δ in units of parts per million (ppm) relative to tetramethylsilane (TMS) where $\delta_{TMS} = 0.00$ ppm. The multiplicity of each signal is indicated by: s (singlet); br s (broad singlet); d (doublet); dd (doublet of doublets); m (multiplet). ^{13}C NMR spectra were recorded at 75.5 MHz

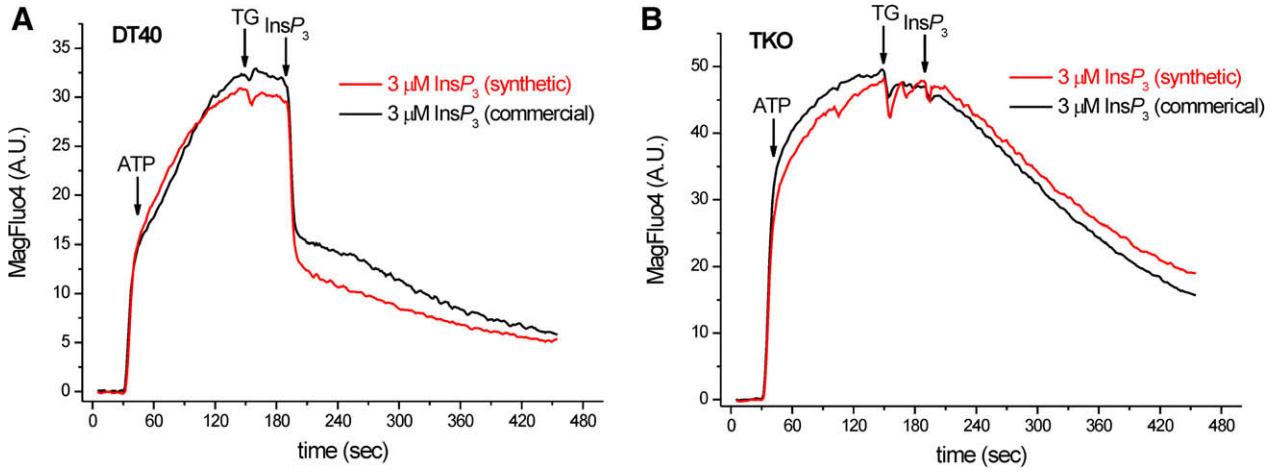


Figure 3. (A) The synthetic InsP_3 mobilises Ca^{2+} from the non-mitochondrial Ca^{2+} stores in permeabilised DT40 cells. A typical experiment showing ER Ca^{2+} levels measured by MagFluo4-fluorescence detection as a function of time using FlexStation 3. ATP was added to initiate Ca^{2+} uptake into the ER. After reaching steady-state ER Ca^{2+} levels, thapsigargin (TG) was added to block SERCA Ca^{2+}-uptake activity and 30 s later, cells were challenged with synthetic or commercial InsP_3 (3 μM) to provoke Ca^{2+} release from the ER, which is observed as an immediate drop in the MagFluo4 signal. (B) The synthetic InsP_3 does not mobilise Ca^{2+} from the non-mitochondrial Ca^{2+} stores in permeabilised InsP_3R-deficient TKO cells. A similar experiment to that described in (A) was performed, except that DT40 cells lacking all three InsP_3R isoforms were used. Addition of synthetic or commercial InsP_3 (3 μM) does not cause Ca^{2+} release from the ER in TKO cells.

or at 100 MHz using the DEPT Q pulse sequence with broadband proton decoupling and internal deuterium lock. The chemical shift data for each signal are given as δ in units of ppm relative to TMS where $\delta_{TMS} = 0.00$ ppm. ^{1}H and ^{13}C spectra were assigned using 2D NMR experiments including COSY, HSQC, HMBC and DEPT Q. Identical proton coupling constants (*J*) are averaged in each spectrum and reported to the nearest 0.1 Hz. ^{31}P NMR spectra were recorded at 121 MHz using broadband proton decoupling and internal deuterium lock. The chemical shift data for each signal are given as δ in units of ppm relative to an external standard of 85% H_3PO_4. Low and high resolution mass spectra were recorded on a Micromass LCT spectrometer using electrospray ionisation in either positive or negative polarity (ES^+ or ES^-). Certain samples were submitted to the National Mass Spectrometry Centre, Swansea. *m/z* values are reported in Daltons and followed by their percentage abundance in parentheses. Microanalyses were obtained on a Carlo Erber EA1110 analyser by the St Andrews University microanalysis service. IR spectra were recorded on a Perkin–Elmer GX FT-IR spectrometer as thin films between sodium chloride disks or as potassium bromide disks as indicated. Absorption maxima are reported in wavenumbers (cm^{-1}). Melting points were determined on a Kofler hot stage or an Electrothermal 9100 and are uncorrected. Optical rotations were measured on a Perkin–Elmer 341 or 241 polarimeters using cells with a path length of 1 dm. The concentration (*c*) is expressed in g/100 mL (equivalent to g/0.1 dm^3). Specific rotations are denoted as $[\alpha]_D^T$ and are given in implied units of 10^{-1} deg cm^2 g^{-1} (*T* = ambient temperature in °C). Analytical thin-layer chromatography (TLC) was carried out on Merck Silica Gel 60 F_{254} pre-coated aluminium-backed plates. Visualisation of the plates was achieved using a UV lamp (λ_{max} 254 or 365 nm) or thermal development after dipping in either an ethanolic solution of phosphomolybdic acid or an ethanolic solution of 4-anisaldehyde, sulfuric acid and acetic acid. Flash column chromatography was carried out using Merck Silica Gel 60 (240–400 mesh), under a positive pressure of compressed air. Anhydrous CH_2Cl_2, THF, diethyl ether and hexane were obtained using a MBRAUN GmbH MB SPS-800 solvent purification system. Anhydrous DMF was purchased from Sigma Aldrich, UK and used without further purification. Where appropriate and if not stated otherwise, all non-aqueous reactions were performed under an inert atmosphere of nitrogen or argon in flame-dried glassware, using a vacuum manifold with nitrogen or argon passed through 4 Å molecular sieves and self-indicating silica gel. In vacuo refers to the use of a rotary evaporator attached to a diaphragm pump. Hexane refers to a mixture of hexanes and brine refers to a saturated aqueous solution of sodium chloride.

4.2. (+)-Methyl 4,6-*O*-anisylidene-α-D-glucopyranoside 2

Methyl 4,6-*O*-anisylidene-α-D-glucopyranoside **2** was synthesised in a manner similar to that described by Hirama and co-workers,[48] affording the desired compound (46.0 g, 58%) as a colourless solid: R_f 0.32 (ethyl acetate); $[\alpha]_D^{20} = +104.8$ (*c* 1.00, $CHCl_3$) {lit.[49] $[\alpha]_D^{20} = +104.7$ (*c* 0.7, $CHCl_3$)}; mp 193–195 °C (from ethyl acetate) [lit.[49] mp 195 °C]; ν_{max} (KBr disc)/cm^{-1} 3373 (s), 2939 (s), 2868 (s), 1615 (s), 1588 (m), 1518 (s), 1459 (s), 1423 (s), 1304 (s), 1036 (s), 931 (s), 890 (m) and 809 (s); ^{1}H NMR (300 MHz; $CDCl_3$) δ 7.45–7.40 (2H, m, Ar ring), 6.91–6.88 (2H, m, Ar ring), 5.49 (1H, s, C*H*-7), 4.79 (1H, d, *J* 4.0, *H*-1), 4.30–4.26 (1H, m, *H*-5), 3.92 (1H, ddd, *J* 9.5, 9.2, 1.5, *H*-3), 3.80 (3H, s, Ar OCH_3), 3.84–3.76 (1H, m, CH_{eq}-6), 3.73 (1H, dd, *J* 10.3, 10.1, CH_{ax}-6), 3.63 (1H, ddd, *J* 9.3, 9.2, 4.0, *H*-2), 3.47 (1H, dd, *J* 9.5, 9.2, *H*-4), 3.46 (1H, s, OCH_3), 2.88 (1H, d, *J* 1.5, O*H*-3) and 2.39 (1H, d, *J* 9.3, O*H*-2); ^{13}C NMR (100 MHz; $CDCl_3$) δ 160.3 (*C*-4 Ar), 129.5 (*C*-1 Ar), 127.6 (*C*-2,6 Ar ring), 113.7 (*C*-3,5 Ar ring), 101.9 (*C*-7), 99.9 (*C*-1), 80.9 (*C*-5), 72.9 (*C*-3), 71.8 (*C*-2), 68.9 (CH_2-6), 62.4 (*C*-4), 55.6 (Ar OCH_3) and 55.3 (OCH_3); HRMS *m/z* (ES^+) [Found: $(M+Na)^+$ 335.1101, $C_{15}H_{20}O_7Na$ requires M^+, 335.1107]; *m/z* (ES+) 335 ($[M+Na]^+$, 100%); Anal. Calcd for $C_{15}H_{20}O_7$: C, 57.69; H, 6.45. Found: C, 57.95; H, 6.37. The data are in good agreement with the literature values.[49,50]

4.3. (+)-Methyl 2-*O*-benzyl-4,6-*O*-anisylidene-α-D-glucopyranoside 3 and (+)-methyl 3-*O*-benzyl-4,6-*O*-anisylidene-α-D-glucopyranoside

A mixture of (+)-methyl 4,6-*O*-anisylidene-α-D-glucopyranoside **2** (15.0 g, 48.0 mmol), di-*n*-butyl tin oxide (13.15 g, 52.8 mmol), tetrabutylammonium bromide (15.47 g, 52.8 mmol) and benzyl bromide (24.64 g, 17.13 mL, 144.1 mmol) were suspended in acetonitrile (400 mL). The suspension was heated at reflux via a Soxhlet thimble filled with 3 Å molecular sieves for 18 h. The mixture was cooled to rt and the volatile components were removed in vacuo providing a colourless residue, which was partitioned between water (250 mL) and ethyl acetate (250 mL). The layers were separated and the aqueous layer was extracted with ethyl acetate (3 × 250 mL). The combined organic phases were washed with brine (200 mL), dried over magnesium sulfate, filtered and concentrated in vacuo. Purification by silica gel chromatography, eluting with hexane, ethyl acetate and triethylamine (70:29:1, 65:34:1, 60:39:1), yielded (+)-methyl 2-*O*-benzyl-4,6-*O*-anisylidene-α-D-glucopyranoside **3** (10.39 g, 54%) as a colourless crystalline solid: R_f 0.45 (hexane/ethyl acetate 1:1); $[\alpha]_D^{20} = +32.0$ (*c* 1.00, $CHCl_3$) {lit.[39] $[\alpha]_D^{20} = +31.9$ (*c* 1.30, $CHCl_3$)}; mp 136–138 °C (from ethyl acetate) [lit.[39] mp 135–136.5 °C]; ν_{max} (KBr disc)/cm^{-1} 3475 (s), 2919 (w), 1613 (s), 1518 (s), 1365 (m), 1250 (s), 1089 (s), 1040 (s) and 990 (s); ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.45–7.29 (7H, m, Ar C*H*), 6.92–6.86 (2H, m, *H*-3,5 $ArOCH_3$ ring), 5.48 (1H, s, C*H*-7), 4.80 (1H, d, *J* 12.2, CH_AH_BPh), 4.71 (1H, d, *J* 12.2, CH_AH_BPh), 4.62 (1H, d, *J* 3.6, *H*-1), 4.25 (1H, dd, *J* 10.1, 4.7, CH_{eq}-6), 4.15 (1H, ddd, *J* 9.0, 9.0, 1.4 *H*-3), 3.84–3.77 (1H, m, *H*-5), 3.80 (3H, s, Ar OCH_3), 3.69 (1H, dd, *J* 10.2, 10.1, CH_{ax}-6), 3.51–3.45 (2H, m, *H*-2 and *H*-4), 3.38 (1H, s, OCH_3) and 2.66 (1H, d, *J* 1.4, O*H*); ^{13}C NMR (100 MHz; $CDCl_3$) δ 160.2 (*C*-4 $ArOCH_3$ ring), 138.0 (*C*-1 Ph), 129.7 (*C*-1 $ArOCH_3$ ring), 128.6 (Ph), 128.2 (Ph), 127.7 (*C*-2,6 $ArOCH_3$ ring), 113.7 (*C*-3,5 $ArOCH_3$ ring), 101.9 (*C*-7), 98.7 (*C*-1), 81.3 (*C*H), 79.6 (*C*H), 73.4 (CH_2Ph), 70.3 (*C*-3), 69.0 (CH_2-6), 62.0 (*C*-5), 55.4 (Ar OCH_3) and 55.3 (OCH_3); HRMS *m/z* (ES^+) [Found: $(M+Na)^+$ 425.1570. $C_{22}H_{26}O_7Na^+$ requires M^+, 425.1576]; *m/z* (ES^+) 425 ($[M+Na]^+$, 100%); Anal. Calcd for $C_{22}H_{26}O_7$: C, 65.66; H, 6.51. Found: C, 65.96; H, 6.80. Further elution provided (+)-methyl 3-*O*-benzyl-4,6-*O*-anisylidene-α-D-glucopyranoside (2.80 g, 15%) as a colourless amorphous solid: R_f 0.29 (hexane/ethyl acetate 1:1); $[\alpha]_D^{20} = +76.6$ (*c* 1.00, $CHCl_3$) {lit.[39] $[\alpha]_D^{20} = +76.2$ (*c* 1.70, $CHCl_3$)}; mp 177–178 °C (from ethyl acetate) [lit.[39] 176–178 °C]; ν_{max} (KBr disc)/cm^{-1} 3449 (s), 1618 (m), 1517 (m), 1370 (m), 1252 (m), 1076 (s); ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.45–7.27 (7H, m, Ar C*H*), 6.94–6.89 (2H, m, *H*-3,5 $ArOCH_3$ ring), 5.54 (1H, s, C*H*-7), 4.96 (1H, d, *J* 11.6, CH_AH_BPh), 4.82 (1H, d, *J* 3.8, *H*-1), 4.79 (1H, d, *J* 11.6 CH_AH_BPh), 4.29 (1 H, dd, *J* 9.6, 4.5, H_{eq}-6), 3.87–3.71 (7H, m, Ar OCH_3, *H*-2, *H*-3, *H*-4 and *H*-5) 3.64 (1H, dd, *J* 9.6, 9.2, H_{ax}-6), 3.46 (3H, s, OCH_3) and 2.33 (1H, d, *J* 7.4, O*H*); ^{13}C NMR (100 MHz; $CDCl_3$) δ 160.0 (*C*-4 $ArOCH_3$ ring), 138.5 (*C*-1 Ph), 129.9 (*C*-1 $ArOCH_3$ ring), 128.4 (Ph), 128.0 (Ph), 127.7 (*C*-4 Ph), 127.4 (*C*-2,6 $ArOCH_3$ ring), 113.6 (*C*-3,5 $ArOCH_3$ ring), 101.3 (*C*-7), 99.9 (*C*-1), 81.9 (*C*H), 78.9 (*C*H), 74.8 (CH_2Ph), 72.4 (*C*H), 69.0 (CH_2-6), 62.6 (*C*H), 55.4 (Ar OCH_3) and 55.3 (OCH_3); HRMS *m/z* (ES^+) [Found: $(M+Na)^+$ 425.1582. $C_{22}H_{26}O_7Na$ requires M^+, 425.1576]; *m/z* (ES^+) 425 ($[M+Na]^+$, 100%); Anal. Calcd for $C_{22}H_{26}O_7$: C, 65.66; H, 6.51. Found: C, 65.82; H, 6.45. The data are in good agreement with the literature values.[39]

4.4. (−)-Methyl 2-*O*-benzyl-3-*O*-triisopropylsilyl-4,6-*O*-anisylidene-α-D-glucopyranoside 4

To a solution of (+)-methyl-2-*O*-benzyl-4,6-*O*-anisylidene-α-D-glucopyranoside **3** (17.61 g, 43.8 mmol) in CH_2Cl_2 (300 mL) were added a mixture of triethylamine (17.70 g, 24.4 mL, 175.0 mmol) and TIPSOTf (20.10 g, 17.7 mL, 65.6 mmol) via a pressure equalised dropping funnel over a period of 20 min. The solution was stirred for 2.5 h at rt, turning from colourless to pale orange. The reaction was quenched by the addition of aqueous saturated sodium bicarbonate solution (200 mL). The layers were separated and the aqueous phase was extracted with CH_2Cl_2 (2 × 300 mL). The combined organic phases were washed with brine (200 mL), dried over magnesium sulfate, filtered and concentrated in vacuo to give an orange oil. Purification by silica gel chromatography, eluting with hexane and diethyl ether (90:10), provided *(−)-methyl 2-O-benzyl-3-O-triisopropylsilyl-4,6-O-anisylidene-α-D-glucopyranoside* **4** (22.46 g, 92%) as a colourless oil: R_f 0.25 (hexane/diethyl ether 80:20); $[\alpha]_D^{20} = -13.5$ (*c* 0.97, $CHCl_3$); ν_{max} (KBr disc)/cm^{-1} 2942 (s), 2865 (s), 1616 (m), 1518 (s), 1375 (m), 1303 (m), 1250 (s), 1054 (s), 992 (s) and 831 (s); ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.41–7.28 (7H, m, Ar C*H*), 6.89–6.87 (2H, m, *H*-3,5 $ArOCH_3$ ring), 5.40 (1H, s, C*H*-7), 4.84 (1H, d, *J* 12.3, CH_AH_BPh), 4.60 (1H, d, *J* 12.3, CH_AH_BPh), 4.47 (1H, d, *J* 3.7, *H*-1), 4.26 (1H, dd, *J* 8.9, 8.9, *H*-3), 4.20 (1H, dd, *J* 10.1, *J* 4.7, H_{eq}-6), 3.81 (3H, s, Ar OCH_3), 3.81–3.78 (1H, m, *H*-5), 3.66 (1H, dd, *J* 10.2, 10.1, H_{ax}-6), 3.42–3.36 (2H, m, *H*-2 and *H*-4), 3.33 (3H, s, OCH_3) and 1.13–0.99 (21H, m, TIPS); ^{13}C NMR (100 MHz; $CDCl_3$) δ 160.1 (*C*-4 $ArOCH_3$ ring), 138.5 (*C*-1 Ph), 129.9 (*C*-1 $ArOCH_3$ ring), 128.4 (Ph), 128.2 (Ph), 127.9 (*C*-4 Ph), 127.7 (*C*-2,6 $ArOCH_3$ ring), 113.4 (*C*-3,5 $ArOCH_3$ ring), 102.1 (*C*-7), 99.3 (*C*-1), 82.7 (*C*H), 80.8 (*C*H), 73.8 (CH_2Ph), 71.8 (*C*H), 69.1 (CH_2-6), 62.6 (*C*H), 55.3 (2 × OCH_3), 18.2 (CH_3 TIPS) and 12.8 (*C*H TIPS); HRMS *m/z* (ES^+) [Found: $(M+Na)^+$ 581.2919. $C_{31}H_{46}O_7SiNa$ requires M^+, 581.2911]; *m/z* (ES^+) 581 ($[M+Na]^+$, 100%); Anal. Calcd for $C_{31}H_{46}O_7Si$: C, 66.63; H, 8.30. Found: C, 66.36; H, 8.65.

4.5. (+)-Methyl 2-*O*-benzyl-4-*O*-(4-methoxybenzyl)-3-*O*-triisopropylsilyl-α-D-glucopyranoside 5

To a solution of (−)-methyl 2-*O*-benzyl-3-*O*-triisopropylsilyl-4,6-*O*-anisylidene-α-D-glucopyranoside **4** (22.46 g, 40.2 mmol) dissolved in CH_2Cl_2 (400 mL) at rt was added a solution of DIBAL-H (1.0 M, 201 mL, 201.0 mmol) in hexane dropwise, via a pressure equalised dropping funnel, over a period of 30 min. The colourless solution was stirred for an additional 40 min at rt. The solution was then cooled to −10 °C and the reaction was quenched by the sequential, dropwise, addition of water (8.04 mL), aqueous sodium hydroxide solution (15% w/v, 8.04 mL) and a further portion of water (20 mL). CAUTION: This quenching procedure may lead to the evolution of hydrogen gas. A thick colourless gel formed, which was stirred for 30 min at 0 °C then filtered through a pad of Celite. The residue was washed with cold CH_2Cl_2 (3 × 500 mL); the resulting filtrates were combined and concentrated in vacuo to provide a pale yellow oil. Purification of the oil by silica gel chromatography, eluting with hexane and diethyl ether (40:60), yielded *(+)-methyl 2-O-benzyl-4-O-(4-methoxybenzyl)-3-O-triisopropylsilyl-α-D-glucopyranoside* **5** (19.82 g, 88%) as a thick colourless oil: R_f 0.35 (hexane/diethyl ether 30:70); $[\alpha]_D^{20} = +64.6$ (*c* 1.56, $CHCl_3$); ν_{max} (thin film)/cm^{-1} 3458 (m), 2942 (s), 2865 (s), 1613 (m), 115 (s), 1464 (m), 1250 (s), 1158 (m), 1104 (s); ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.40–7.24 (7H, m, Ar C*H*), 6.91–6.86 (2H, m, *H*-3,5 $ArOCH_3$ ring), 4.85 (1H, d, *J* 10.7, $CH_AH_BPhOCH_3$), 4.65 (1H, d, *J* 11.8, CH_AH_BPh), 4.56 (1H, d, *J* 10.7, $CH_AH_BPhOCH_3$), 4.54 (1H, d, *J* 3.4, *H*-1), 4.52 (1H, d, *J* 11.8, CH_AH_BPh), 4.22 (1H, dd, *J* 9.1, 8.9 *H*-3), 3.81 (3H, s, Ar OCH_3), 3.76–3.56 (3H, m, *H*-5, H_{eq}-6, H_{ax}-6), 3.41 (1H, dd, *J* 9.9, 8.9, *H*-4), 3.33 (1H, dd, *J* 9.1, 3.4, *H*-2), 3.21 (3H, s, OCH_3), 1.68–1.64 (1H, m, O*H*) and 1.20–1.05 (21H, m, TIPS); ^{13}C NMR (100 MHz; $CDCl_3$) δ 159.2 (*C*-4 $ArOCH_3$ ring), 138.5 (*C*-1 Ph), 130.7 (*C*-1 $ArOCH_3$ ring), 129.3 (*C*-2,6 $ArOCH_3$ ring), 128.2 (Ph), 128.0 (Ph), 127.7 (*C*-4 Ph), 113.8 (*C*-3,5 $ArOCH_3$ ring), 97.6 (*C*-1), 81.3 (*C*-2), 78.9 (*C*-4), 74.4 (*C*-3), 74.3 (*C*H$_2$Ph), 72.6 (CH_2PhOCH_3), 70.4 (*C*-5), 62.0 (CH_2-6), 55.2 (Ar OCH_3), 54.9 (OCH_3), 18.3 (*C*H$_3$ TIPS) and 13.6 (*C*H TIPS); HRMS *m/z* (ES^+) [Found: $(M+Na)^+$ 583.3071. $C_{31}H_{48}O_7SiNa$ requires M^+, 583.3067]; *m/z* (ES^+) 583 ($[M+Na]^+$, 100%); Anal. Calcd for $C_{31}H_{48}O_7Si$: C, 66.39; H, 8.63. Found: C, 66.62; H, 8.71.

4.6. Methyl 2-*O*-benzyl-4-*O*-(4-methoxybenzyl)-3-*O*-triisopropylsilyl-α-D-gluco-hexadialdo-1,5-pyranoside 6

A solution of (+)-methyl 2-*O*-benzyl-4-*O*-(4-methoxybenzyl)-3-*O*-triisopropylsilyl-α-D-glucopyranoside **5** (19.82 g, 35.3 mmol) in CH_2Cl_2 (300 mL) was treated with Dess Martin Periodinane (22.46 g, 53.0 mmol) in a single portion. The suspension was stirred at rt for 1.5 h, after which time TLC analysis indicated consumption of the starting material. The suspension was diluted with diethyl ether (200 mL) and aqueous saturated sodium bicarbonate solution (200 mL), then sodium thiosulfate (134.8 g) was added. The biphasic mixture was stirred vigorously for 30 min until two clear layers were observed. The layers were separated and the aqueous phase was re-extracted with diethyl ether (2 × 400 mL). The combined organic phases were then washed with aqueous saturated sodium bicarbonate solution (300 mL) and water (400 mL), dried over magnesium sulfate, filtered and concentrated in vacuo providing a pale yellow oil. Purification of the oil by silica gel chromatography, eluting with hexane and diethyl ether (40:60), yielded *methyl 2-O-benzyl-4-O-(4-methoxybenzyl)-3-O-triisopropylsilyl-α-D-glucohexadialdo-1,5-pyranoside* **6** (16.26 g, 82%) as a colourless oil: R_f 0.47 (hexane/ethyl acetate 50:50); ν_{max} (thin film)/cm^{-1} 2943 (s), 2865 (s), 1742 (m), 1613 (m), 1515 (s), 1464 (m), 1250 (s), 1051 (s); ^{1}H NMR (400 MHz; $CDCl_3$) δ 9.59 (1H, d, *J* 0.6, C*H*O), 7.40–7.21 (7H, m, Ar C*H*), 6.90–6.86 (2H, m, *H*-3,5 $ArOCH_3$ ring), 4.82 (1H, d, *J* 10.8, $CH_AH_BPhOCH_3$), 4.67 (1H, d, *J* 11.9, CH_AH_BPh), 4.64 (1H, d, *J* 3.3, *H*-1), 4.52 (1H, d, *J* 11.9, CH_AH_BPh), 4.51 (1H, d, *J* 10.8, $CH_AH_BPhOCH_3$), 4.28 (1H, dd, *J* 8.8, 8.0, *H*-3), 4.16 (1H, d, *J* 9.9, *H*-5), 3.81 (3H, s, Ar OCH_3), 3.49 (1H, dd, *J* 9.9, 8.0, *H*-4), 3.33 (1H, dd, *J* 8.8, 3.3, *H*-2), 3.24 (3H, s, OCH_3) and 1.18–1.02 (21H, m, TIPS); ^{13}C NMR (100 MHz; $CDCl_3$) δ 198.0 (*C*HO), 159.4 (*C*-4 $ArOCH_3$ ring), 138.3 (*C*-1 Ph), 129.7 (*C*-1 $ArOCH_3$ ring), 129.5 (*C*-2,6 $ArOCH_3$ ring), 128.3 (Ph), 128.0 (Ph), 127.8 (*C*-4 Ph), 113.8 (*C*-3,5 $ArOCH_3$ ring), 97.9 (*C*-1), 82.3 (*C*H), 79.1 (*C*H), 74.9 (*C*H), 74.3 (*C*H$_2$Ph), 74.0 (*C*H), 72.8 (CH_2PhOCH_3), 55.6 (Ar OCH_3), 55.3 (OCH_3), 18.3 (*C*H$_3$ TIPS) and 13.4 (*C*H TIPS); HRMS *m/z* (ES^+) [Found: $(M+Na)^+$ 581.2902. $C_{31}H_{46}O_7SiNa$ requires M^+, 581.2911]; *m/z* (ES^+) 581 ($[M+Na]^+$, 100%). This material was used immediately in the next step.

4.7. (−)-Methyl 6-*O*-acetyl-2-*O*-benzyl-4-*O*-(4-methoxybenzyl)-3-*O*-triisopropylsilyl-α-D-glucohex-5-enopyranoside 7

To a solution of methyl 2-*O*-benzyl-4-*O*-(4-methoxybenzyl)-3-*O*-triisopropylsilyl-α-D-gluco-hexadialdo-1,5-pyranoside **6** (16.26 g, 29.1 mmol) in acetonitrile (300 mL) was added acetic anhydride (8.91 g, 6.41 mL, 87.3 mmol), potassium carbonate (20.11 g, 145.5 mmol) and DMAP (355 mg, 2.9 mmol). The stirred suspension was heated to 90 °C for 1 h, after which time TLC analysis showed consumption of starting material. The mixture was cooled to RT and the volatile components were removed in vacuo providing a yellow residue. This residue was partitioned between water (400 mL) and CH_2Cl_2 (400 mL). The layers were separated and the aqueous layer was re-extracted with CH_2Cl_2 (3 × 400 mL). The combined organic layers were washed with brine

(400 mL), dried over magnesium sulfate, filtered and concentrated in vacuo to give a light brown oil. Purification of this oil by silica gel chromatography, eluting with hexane and diethyl ether (75:25), provided *(−)-methyl 6-O-acetyl-2-O-benzyl-4-O-(4-methoxybenzyl)-3-O-triisopropylsilyl-α-D-glucohex-5-enopyranoside* **7** (11.42 g, 65%) as a colourless oil: R_f 0.18 (hexane/diethyl ether 75:25); $[\alpha]_D^{20} = -8.6$ (*c* 1.32, $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.43–7.28 (7H, m, Ar C*H*), 7.08 (1H, d, *J* 1.5, C*H*OAc), 6.92–6.88 (2H, m, *H*-3,5 $ArOCH_3$ ring), 4.78 (1H, d, *J* 12.0, CH_AH_BPh), 4.68 (1H, d, *J* 3.0, *H*-1), 4.64 (1H, d, *J* 10.9, $CH_AH_BPhOCH_3$), 4.61 (1H, d, *J* 12.0, CH_AH_BPh), 4.58 (1H, d, *J* 10.9, $CH_AH_BPhOCH_3$), 4.19 (1H, dd, *J* 8.0, 7.1, *H*-3), 3.83 (3H, s, Ar OCH_3), 3.76 (1H, dd, *J* 7.1, 1.5, *H*-4), 3.46 (1H, dd, *J* 8.0, 3.0, *H*-2), 2.17 (3H, s, OCH_3) and 1.10–1.03 (21 H, m, TIPS); ^{13}C NMR (100 MHz; $CDCl_3$) δ 167.4 (*C*=O), 159.2 (*C*-4 Ar-OCH_3 ring), 138.4 (*C*-1 Ph), 135.1 (*C*-5), 129.9 (*C*-1 $ArOCH_3$ ring), 129.4 (*C*-2,6 $ArOCH_3$ ring), 128.3 (Ph), 128.1 (Ph), 127.8 (*C*-4 Ph), 123.1 (*C*HOAc), 113.7 (*C*-3,5 $ArOCH_3$ ring), 100.0 (*C*-1), 80.2 (*C*-2), 78.5 (*C*-4), 73.4 (*C*-3), 73.3 (CH_2Ph), 72.6 (CH_2PhOCH_3), 56.3 (OCH_3), 55.3 (Ar OCH_3), 20.6 (CH_3 Ac), 18.2 (CH_3 TIPS) and 12.9 (*C*H TIPS); HRMS *m/z* (ES^+) [Found: $(M+Na)^+$ 623.3018. $C_{33}H_{48}O_8Si$-Na requires M^+, 623.3016]; *m/z* (ES^+) 623 ($[M+Na]^+$, 100%); Anal. Calcd for $C_{33}H_{48}O_8Si$: C, 65.97; H, 8.05. Found: C, 65.88; H, 8.45.

4.8. (−)-3-*O*-Benzyl-2-hydroxy-5-*O*-(4-methoxybenzyl)-4-*O*-triisopropylsilyl-6-oxocyclohexyl acetate 8

To a solution of (−)-methyl 6-*O*-acetyl-2-*O*-benzyl-4-*O*-(4-methoxybenzyl)-3-*O*-triisopropylsilyl-α-D-gluco-hex-5-enopyranoside **7** (11.42 g, 19.0 mmol) in acetone (400 mL) and water (260 mL) was added mercuric acetate (30.29 g, 95.1 mmol) in a single portion. The cloudy solution immediately turned to a thick yellow suspension. The suspension was stirred for 3 h at rt, after which time TLC analysis indicated consumption of the starting material. The suspension was diluted with brine (130 mL), turning the suspension to a slightly cloudy colourless solution after 15 min. The mixture was then stirred for an additional 24 h. The mixture was diluted with water (200 mL) and extracted with $CHCl_3$ (400 mL). The aqueous phase was re-extracted with a further portion of $CHCl_3$ (400 mL). The combined organic layers were washed with brine (200 mL), dried over magnesium sulfate, filtered and concentrated in vacuo to give a thick colourless oil. Purification of the oil by silica gel chromatography, eluting with hexane and diethyl ether (75:25, 70:30, 60:40, 0:100), yielded a colourless solid. Crystallisation from hexane and diethyl ether yielded *(−)-3-O-benzyl-2-hydroxy-5-O-(4-methoxybenzyl)-4-O-triisopropylsilyl-6-oxocyclohexyl acetate* **8** (3.85 g, 35%) as colourless needles: R_f 0.51 (hexane/ethyl acetate 50:50); $[\alpha]_D^{20} = -28.9$ (*c* 3.02, $CHCl_3$); mp 102–103 °C (from hexane/diethyl ether); ν_{max} (thin film)/cm^{-1} 3490 (s), 2943 (s), 2866 (s), 1756 (s), 1737 (s), 1611 (m), 1514 (m), 1467 (m), 1367 (m), 1242 (s), 1145 (s), 1094 (m), 1073 (m), 1027 (m) and 885 (m); ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.41–7.32 (7H, m, Ar C*H*), 6.90–6.86 (2H, m, *H*-3,5 $ArOCH_3$ ring), 5.28–5.25 (1H, m, *H*-1), 4.79 (1H, d, *J* 9.7, $CH_AH_BPhOCH_3$), 4.78 (1H, d, *J* 11.8, CH_AH_BPh), 4.68 (1H, d, *J* 11.8, CH_AH_BPh), 4.40 (1H, d, *J* 9.7, $CH_AH_BPhOCH_3$), 4.33 (1H, dd, *J* 4.2, 2.7, *H*-2), 4.28 (1H, dd, *J* 8.8, 8.6, *H*-4), 4.00 (1H, dd, *J* 8.8, 1.0, *H*-5), 3.83 (3H, s, OCH_3), 3.78 (1H, dd, *J* 8.6, 2.7, *H*-3), 2.45 (1H, m, O*H*), 2.26 (2H, s, CH_3) and 1.14–1.01 (21 H, m TIPS); ^{13}C NMR (100 MHz; $CDCl_3$) δ 198.3 (*C*-6 *C*=O), 169.6 (*C*=O), 159.2 (*C*-4 $ArOCH_3$ ring), 137.4, 130.0, 129.6 (*C*-1 $ArOCH_3$ ring), 128.6, 128.1, 127.8 (*C*-2,6 $ArOCH_3$ ring), 113.5 (*C*-3,5 $ArOCH_3$ ring), 83.7 (*C*-5), 80.9 (*C*-3), 74.9 (*C*-4), 74.9 (*C*-1), 72.9 (CH_2Ph), 72.7 (CH_2ArOCH_3), 68.6 (*C*-2), 55.3 (OCH_3), 20.6 (CH_3), 18.2 (CH_3 TIPS) and 13.0 (*C*H TIPS); HRMS *m/z* (ES^+) [Found: $(M+Na)^+$ 609.2859. $C_{32}H_{46}O_8SiNa$ requires M^+, 609.2860]; *m/z* (ES^+) 609 ($[M+Na]^+$, 100%); Anal. Calcd for $C_{32}H_{46}O_8Si$: C, 65.50; H, 7.90. Found: C, 65.32; H, 8.29.

4.9. (+)-D-1-*O*-Acetyl-3-*O*-benzyl-5-*O*-(4-methoxybenzyl)-4-*O*-triisopropylsilyl-*myo*-inositol 9

A mixture of acetic acid (36.5 mL) and acetonitrile (36.5 mL) was cooled to −40 °C and to this was added tetramethylammonium triacetoxyborohydride (8.63 g, 32.8 mmol). To the resulting slurry was added a solution of (−)-3-*O*-benzyl-2-hydroxy-5-*O*-(4-methoxybenzyl)-4-*O*-triisopropylsilyl-6-oxocyclohexyl acetate **8** (3.85 g, 6.56 mmol) in acetonitrile (23.9 mL) via cannula and the mixture stirred for 10 min at −40 °C. The mixture was then allowed to warm to rt and was stirred for a further 18 h, by which time a clear solution had formed. The reaction was quenched by the addition of aqueous saturated Rochelle's Salt solution (100 mL). The resulting emulsion was stirred vigorously for 1 h and then diluted with CH_2Cl_2 (250 mL). The layers were separated and the aqueous phase was re-extracted with CH_2Cl_2 (2 × 250 mL). The combined organic phases were washed with brine (200 mL), dried over magnesium sulfate, filtered and concentrated in vacuo providing a brown oil. The excess AcOH was then removed by azeotropic distillation with cyclohexane (3 × 100 mL), resulting in a brown gum. Purification of the gum by silica gel chromatography, eluting with hexane and diethyl ether (50:50, 40:60, 0:100), yielded *(+)-D-1-O-acetyl-3-O-benzyl-5-O-(4-methoxybenzyl)-4-O-triisopropylsilyl-myo-inositol* **9** (3.42 g, 89%) as a colourless oil: R_f 0.39 (hexane/ethyl acetate 50:50); $[\alpha]_D^{20} = +17.1$ (*c* 1.00, $CHCl_3$); ν_{max} (thin film)/cm^{-1} 3449 (m), 3012 (m), 2945 (m), 2867 (w), 1735 (m), 1612 (w), 1514 (m), 1215 (s), 768 (s); ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.39–7.26 (7H, m, Ar C*H*), 6.90–6.89 (2H, m, *H*-3,5 $ArOCH_3$ ring), 4.87 (1H, d, *J* 11.4, $CH_AH_BPhOCH_3$), 4.75 (1H, dd, *J* 9.8, 2.2, *H*-1), 4.70 (1H, d, *J* 11.4, $CH_AH_BPhOCH_3$), 4.61–4.55 (2H, m, CH_2Ph), 4.31–4.28 (1H, m, *H*-2), 4.15 (1H, dd, *J* 8.9, 8.8, *H*-4), 4.05 (1H, ddd, *J* 9.8, 9.3, 3.1, *H*-6), 3.80 (3H, s, Ar OCH_3), 3.41 (1H, dd, *J* 8.9, 2.8, *H*-3), 3.26 (1H, dd, *J* 9.3, 8.8, *H*-5), 2.28 (1H, s, O*H*-2), 2.14 (3H, s, $COCH_3$), 1.64 (1H, d, *J* 3.1, O*H*-6) and 1.16–1.03 (21H, m, TIPS); ^{13}C NMR (100 MHz; $CDCl_3$) δ 170.9 (*C*=O), 159.2 ($COCH_3$), 137.6, 131.0, 129.2 (*C*H-2,6 $ArOCH_3$ ring), 128.5 (*C*H Ph), 128.0 (*C*H Ph), 127.6 (*C*H Ph), 114.0 (*C*H-3,5 $ArOCH_3$ ring), 84.2 (*C*-5), 81.4 (*C*-3), 74.9 (CH_2Ph), 73.5 (*C*-4), 73.2 (*C*-1), 71.7 (CH_2PhOCH_3), 70.3 (*C*-6), 66.5 (*C*-2), 55.3 (OCH_3), 21.1 ($COCH_3$), 18.2 (CH_3 TIPS) and 13.5 (*C*H TIPS); HRMS *m/z* (ES^+) [Found: $(M+Na)^+$ 611.3012. $C_{32}H_{48}O_8SiNa$ requires M^+, 611.3016]; *m/z* (ES^+) 611 ($[M+Na]^+$, 100%); Anal. Calcd for $C_{32}H_{48}O_8Si$: C, 65.28; H, 8.22. Found: C, 64.99; H, 8.17.

4.10. (−)-D-1-*O*-Acetyl-3-*O*-benzyl-2,6-*O*-bisbenzyloxymethyl-5-*O*-(4-methoxybenzyl)-4-*O*-triisopropylsilyl-*myo*-inositol 10

A mixture of benzyloxymethyl chloride (7.28 g, 6.46 mL, 46.47 mmol) and Hünig's base (20 mL) was stirred for 1 h at rt, forming a light brown precipitate. The precipitate was allowed to settle, then the solution was carefully decanted from the mixture via cannula into a solution of (+)-D-1-*O*-acetyl-3-*O*-benzyl-5-*O*-(4-methoxybenzyl)-4-*O*-triisopropylsilyl-*myo*-inositol **9** (3.42 g, 5.81 mmol) in Hünig's base (40 mL). The resulting solution was heated to 85 °C for 15 h, then allowed to cool to rt. TLC analysis of the mixture indicated consumption of the starting material. The volatile components were removed in vacuo and the resulting brown residue was partitioned between water (250 mL) and ethyl acetate (250 mL). The layers were separated and the aqueous phase re-extracted with ethyl acetate (2 × 250 mL). The combined organic layers were washed with brine (250 mL) dried over magnesium sulfate, filtered and concentrated in vacuo affording a brown oil. Purification of the oil by silica gel chromatography, eluting with hexane and diethyl ether (100:0, 98:2, 96:4, 94:6, 92:8, 80:20), furnished *(−)-D-1-O-acetyl-3-O-benzyl-2,6-O-bisbenzyloxymethyl-5-O-(4-methoxybenzyl)-4-O-triisopropylsilyl-myo-inositol* **10** (3.94 g,

82%) as a colourless gum, which solidified on standing to waxy solid: R_f 0.46 (hexane/diethyl ether 50:50); $[\alpha]_D^{20} = -40.7$ (*c* 0.75, $CHCl_3$); ν_{max} (KBr disc)/cm^{-1} 3415 (m), 2944 (s), 2866 (s), 1741 (s) 1615 (m), 1514 (s), 1458 (m), 1366 (m), 1247 (s), 1119 (s), 1039 (s), 739 (s) and 699 (m); ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.25–7.15 (17H, m, Ar H), 6.77–6.73 (2H, m, *H*-3,5 $ArOCH_3$ ring), 4.87 (1H, d, *J* 6.7, OCH_AH_BO), 4.81–4.65 (8H, m, *H*-1 and 7 × CH_AH_B protons), 4.59 (1H, d, *J* 12.3, CH_AH_BPh), 4.50 (1H, d, *J* 12.3, CH_AH_BPh'), 4.39 (1H, d, *J* 12.3, CH_AH_BPh), 4.38 (1H, d, *J* 11.1, CH_AH_BPh''), 4.31 (1H, dd, *J* 2.1, 2.1, *H*-2), 4.23 (1H, dd, *J* 9.3, 9.0, *H*-4), 4.14 (1H, dd, *J* 9.3, 9.2, *H*-6), 3.71 (3H, s, Ar OCH_3), 3.29 (1H, dd, *J* 9.2, 9.0, *H*-5), 3.27 (1H, dd, *J* 9.3, 2.1, *H*-3), 1.72 (3H, s, $COCH_3$) and 0.96–0.90 (21H, m, TIPS); ^{13}C NMR (100 MHz; $CDCl_3$) δ 170.7 (*C*=O), 158.7 ($COCH_3$), 138.0, 137.8, 137.6, 131.2, 128.4, 128.3 (*C*H-2,6 $ArOCH_3$ ring), 128.3, 128.2, 127.6, 127.5, 127.5, 127.4, 127.4, 113.5 (*C*H-3,5 $ArOCH_3$ ring), 96.2 (OCH_2O), 95.0 (OCH_2O), 84.7 (*C*-5), 81.0 (*C*-3), 77.2 (*C*-6), 75.1 (CH_2Ar), 73.8 (*C*-4), 72.9 (*C*-1), 72.0 (*C*-2), 71.9 (CH_2Ph), 69.7 (CH_2Ph), 69.2 (CH_2Ph), 55.3 (Ar OCH_3), 21.0 ($COCH_3$), 18.2 (CH_3 TIPS) and 13.5 (*C*H TIPS); HRMS *m/z* (ES^+) [Found: $(M+Na)^+$ 851.4155. $C_{48}H_{64}O_{10}SiNa$ requires M^+, 851.4166]; *m/z* (ES^+) 851 ($[M+Na]^+$, 100%); Anal. Calcd for $C_{48}H_{64}O_{10}Si$: C, 69.53; H, 7.78. Found: C, 69.41; H, 7.91.

4.11. (−)-D-3-*O*-Benzyl-2,6-*O*-bisbenzyloxymethyl-5-*O*-(4-methoxybenzyl)-4-*O*-triisopropylsilyl-*myo*-inositol 11

In an open flask, (−)-D-1-*O*-acetyl-3-*O*-benzyl-2,6-*O*-bisbenzyloxymethyl-5-*O*-(4-methoxybenzyl)-4-*O*-triisopropylsilyl-*myo*-inositol **10** (3.48 g, 4.20 mmol) was dissolved in a mixture of methanol (90 mL) and tetrahydrofuran (30 mL). To this solution was added lithium hydroxide (422 mg, 17.63 mmol) in a single portion. The mixture was stirred vigorously at rt for 15 min, after which time TLC analysis indicated consumption of the starting material. The reaction was quenched by the addition of saturated aqueous ammonium chloride solution (20 mL) and the volatile components were removed in vacuo. The resulting residue was partitioned between water (150 mL) and CH_2Cl_2 (150 mL). The layers were separated and the aqueous layer was re-extracted with CH_2Cl_2 (3 × 100 mL). The combined organic phases were washed with brine (100 mL), dried over magnesium sulfate, filtered and concentrated in vacuo, furnishing a colourless oil. Purification of the oil by silica gel chromatography, eluting with hexane and diethyl ether (70:30), afforded *(−)-D-3-O-benzyl-2,6-O-bisbenzyloxymethyl-5-O-(4-methoxybenzyl)-4-O-triisopropylsilyl-myo-inositol* **11** (3.19 g, 97%) as a colourless oil, which solidified on standing to give a waxy solid: R_f 0.37 (hexane/diethyl ether 50:50); $[\alpha]_D^{20} = -17.9$ (*c* 1.47, $CHCl_3$); ν_{max} (thin film)/cm^{-1} 3449 (m), 2942 (s), 2863 (s), 1617 (w), 1514 (s), 1464 (s), 1382 (w), 1245 (s), 1164 (s), 1133 (s), 1069 (s), 1030 (s), 885 (w), 824 (m), 749 (m) and 699 (m); ^{1}H NMR (500 MHz; $CDCl_3$) δ 7.32–7.25 (15H, m, Ar C*H*), 7.24–7.22 (2H, m, *H*-2,6 $ArOCH_3$), 6.86–6.83 (2H, m, *H*-3,5 $ArOCH_3$), 4.92 (1H, d, *J* 6.8, OCH_AH_BO), 4.83 (1H, d, *J* 6.6, OCH_AH_BO'), 4.79 (1H, d, *J* 6.6, OCH_AH_BO'), 4.78 (1H, d, *J* 10.9, $OCH_AH_BPhOCH_3$), 4.77 (1H, d, *J* 6.8, OCH_AH_BO), 4.74 (1H, d, *J* 10.9, $OCH_AH_BPhOCH_3$), 4.73 (1H, d, *J* 11.9, CH_AH_BPh), 4.72 (1H, d, *J* 12.0, CH_AH_BPh'), 4.70 (1H, d, *J* 11.5, CH_AH_BPh''), 4.58 (1H, d, *J* 12.0, CH_AH_BPh'), 4.53 (1H, d, *J* 11.9, CH_AH_BPh), 4.49 (1H, d, *J* 11.5, CH_AH_BPh''), 4.27 (1H, dd, *J* 8.9, 8.6, *H*-4), 4.36 (1H, dd, *J* 2.5, 2.3, H-2), 3.88 (1H, d, *J* 4.8, O*H*-1), 3.86 (1H, dd, *J* 9.1, 8.9, *H*-6), 3.80 (3H, s, OCH_3), 3.53 (1H, ddd, *J* 9.1, 4.8, 2.5, *H*-1), 3.33 (1H, dd, *J* 8.9, 8.6, *H*-5), 3.31 (1H, dd, *J* 8.9, 2.3, *H*-3) and 1.09–0.99 (21H, m, TIPS); ^{13}C NMR (100 MHz; $CDCl_3$) δ 158.8 ($CHOCH_3$), 137.9, 137.8, 137.3, 131.4, 128.6, 128.5 (*C*-2,6 $ArOCH_3$ ring), 128.3, 128.1, 128.0, 127.8, 127.6, 127.6, 127.5, 113.5 (*C*-3,5 $ArOCH_3$ ring), 96.5 (OCH_2O'), 95.5 (OCH_2O), 83.8 (*C*-6), 83.7 (*C*-5), 80.9 (*C*-3), 74.8 (OCH_2Ar), 74.7 (*C*-2), 73.7 (*C*-4), 71.9 (CH_2Ph''), 71.2 (*C*-1), 70.1 (CH_2Ph), 69.5 (CH_2Ph'), 55.3 (OCH_3), 18.3 (CH_3 TIPS) and 13.4 (*C*H TIPS); HRMS *m/z* (ES^+) [Found: $(M+Na)^+$ 809.4079. $C_{46}H_{62}O_9SiNa$ requires M^+, 809.4061]; *m/z* (ES^+) 809 ($[M+Na]^+$, 100%); Anal. Calcd for $C_{46}H_{62}O_9Si$: C, 70.20; H, 7.94. Found: C, 70.21; H, 7.74.

4.12. (+)-D-3-*O*-Benzyl-2,6-*O*-bisbenzyloxymethyl-4-*O*-triisopropylsilyl-*myo*-inositol 12

To a solution of (−)-D-3-*O*-benzyl-2,6-*O*-bisbenzyloxymethyl-5-*O*-(4-methoxybenzyl)-4-*O*-triisopropylsilyl-*myo*-inositol **11** (1.23 g, 1.56 mmol) in CH_2Cl_2 (60 mL) and water (3.3 mL) was added 2,3-dichloro-5,6-dicyanobenzoquinone (780 mg, 3.44 mmol) in a single portion, turning the mixture dark green. After 10 min stirring at rt, the colour changed to orange/yellow and TLC analysis indicated consumption of the starting material. The solution was diluted with aqueous saturated sodium hydrogen carbonate solution (40 mL) and CH_2Cl_2 (80 mL), forming a dark biphasic mixture. The biphasic mixture was filtered through a pad of Celite and the layers were subsequently separated. The aqueous phase was re-extracted with CH_2Cl_2 (3 × 80 mL). The combined organic phases were washed with aqueous saturated sodium hydrogen carbonate solution (100 mL) and brine (100 mL), dried over magnesium sulfate, filtered and concentrated in vacuo affording a pale brown oil. Purification of the oil by silica gel chromatography, eluting with hexane and diethyl ether (60:40), furnished *(+)-D-3-O-benzyl-2,6-O-bisbenzyloxymethyl-4-O-triisopropylsilyl-myo-inositol* **12** (993 mg, 95%) as a colourless oil: R_f 0.21 (hexane/diethyl ether 50:50); $[\alpha]_D^{20} = +3.6$ (*c* 2.45, $CHCl_3$); ν_{max} (thin film)/cm^{-1} 3439 (s), 3065 (m), 3032 (m), 2493 (s), 2866 (s), 1719 (m), 1497 (m), 1455 (s), 1368 (s), 1273 (m), 1025 (s), 884 (s), 826 (s), 737 (s) and 698 (s); ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.30–7.17 (15H, m, Ar H), 4.89 (1H, d, *J* 10.4, OCH_AH_BO), 4.87 (1H, d, *J* 10.4, OCH_AH_BO), 4.85 (1H, d, *J* 6.6, OCH_AH_BO'), 4.68 (1H, d, *J* 6.6, OCH_AH_BO'), 4.65 (1H, d, *J* 11.9, OCH_AH_BPh'), 4.62 (2H, s, OCH_2Ph), 4.59 (1H, d, *J* 11.6, CH_AH_BPh''), 4.51 (1H, d, *J* 11.9, OCH_AH_BPh'), 4.49 (1H, d, *J* 11.6, CH_AH_BPh''), 4.10 (1H, dd, *J* 2.3, 2.3, *H*-2), 4.06 (1H, dd, *J* 9.3, 8.9, *H*-4), 3.60 (1H, dd, *J* 9.2, 9.0, *H*-6), 3.47 (1H, d, *J* 5.6, O*H*-1), 3.44–3.38 (1H, m, *H*-1), 3.32 (1H, ddd, *J* 9.0, 8.9, 2.0, *H*-5), 3.19 (1H, dd, *J* 9.3, 2.3, *H*-3), 3.01 (1H, d, *J* 2.0, O*H*-5) and 1.11–0.95 (21H, m, TIPS); ^{13}C NMR (100 MHz; $CDCl_3$) δ 138.0, 137.6, 137.0, 128.5, 128.4, 128.2, 128.1, 128.0, 127.9, 127.7, 127.6, 127.6, 96.5 (OCH_2O), 95.9 (OCH_2O'), 84.1 (*C*-6), 80.3 (*C*-3), 76.3 (*C*-2), 75.5 (*C*-5), 74.3 (*C*-4), 72.2 (CH_2Ph''), 70.9 (*C*-1), 70.4 (CH_2Ph), 69.8 (CH_2Ph'), 18.3 (CH_3 TIPS) and 13.0 (*C*H TIPS); HRMS *m/z* (ES^+) [Found: $(M+Na)^+$ 689.3475. $C_{38}H_{54}O_8SiNa$ requires M^+, 689.3486]; *m/z* (ES^+) 689 ($[M+Na]^+$, 100%); Anal. Calcd for $C_{38}H_{54}O_8Si$: C, 68.44; H, 8.16. Found: C, 68.02; H, 8.30.

4.13. (+)-D-3-*O*-Benzyl-2,6-*O*-bisbenzyloxymethyl-*myo*-inositol 13

To a solution of (+)-D-3-*O*-benzyl-2,6-*O*-bisbenzyloxymethyl-4-*O*-triisopropylsilyl-*myo*-inositol **12** (947 mg, 1.42 mmol) in CH_2Cl_2 (30 mL) was added a solution of tetrabutylammonium fluoride (1.0 M, 14.2 mL) in tetrahydrofuran. The solution was stirred at rt for 24 h, after which time TLC analysis indicated consumption of the starting material. The reaction was quenched by the addition of saturated aqueous sodium hydrogen carbonate solution (50 mL) and the layers were separated. The aqueous phase was re-extracted with CH_2Cl_2 (4 × 50 mL). The combined organic phases were washed with brine (50 mL), dried over magnesium sulfate, filtered and concentrated in vacuo, providing a brown oil. Purification by silica gel chromatography, eluting with hexane and ethyl acetate (40:60), provided (+)-D-3-*O*-benzyl-2,6-*O*-bisbenzyloxymethyl-*myo*-inositol **13** (655 mg, 90%) as a colourless solid: R_f 0.26 (hexane/ethyl acetate 65:35); $[\alpha]_D^{20} = +27.3$ (*c* 0.11,

$CHCl_3$) {lit.[28] $[\alpha]_D^{24} = +26.8$ (*c* 0.45, $CHCl_3$)}; mp 113–114 °C (from ethyl acetate) [lit.[28] 112 °C]; ν_{max} (thin film)/cm^{-1} 3438 (s), 2899 (m), 1498 (w), 1454 (m), 1378 (m), 1171 (s), 1129 (s), 1105 (s), 1065 (s), 1020 (s), 942 (m), 737 (s) and 698 (s); ^{1}H NMR (500 MHz; $CDCl_3$) δ 7.37–7.28 (15H, m, Ar H), 5.00 (1H, d, *J* 6.8, OCH_AH_BO), 4.98 (1H, d, *J* 7.0, OCH_AH_BO'), 4.92 (1H, d, *J* 7.0, OCH_AH_BO'), 4.82 (1H, d, *J* 6.8, OCH_AH_BO), 4.74 (1H, d, *J* 11.7, CH_AH_BPh), 4.73 (1H, d, *J* 12.0, CH_AH_BPh'), 4.72 (1H, d, *J* 11.9, CH_AH_BPh''), 4.69 (1H, d, *J* 11.7, CH_AH_BPh), 4.64 (1H, s, *J* 12.0, CH_AH_BPh'), 4.62 (1H, d, *J* 11.9, CH_AH_BPh''), 4.25 (1H, dd, *J* 2.4, 2.3, *H*-2), 3.99 (1H, dd, *J* 9.7, 9.3, *H*-4), 3.65 (1H, dd, *J* 9.2, 9.1, *H*-6), 3.66–3.62 (1H, m, O*H*-5), 3.54 (1H, d, *J* 5.6, O*H*-1), 3.51–3.48 (1H, m, *H*-1), 3.43 (1H, dd, J 9.3, 9.1, *H*-5), 3.32 (1H, dd, J 9.7, 2.4, *H*-3) and 2.71 (1H, br s, O*H*-4); ^{13}C NMR (100 MHz; $CDCl_3$) δ 137.6, 137.5, 136.8, 128.6, 128.6, 128.5, 128.1, 128.1, 128.0, 127.9, 127.9, 127.8, 96.6 (OCH_2O'), 96.0 (OCH_2O), 84.6 (*C*-6), 78.9 (*C*-3), 76.0 (*C*-2), 74.0 (*C*-5), 72.4 (*C*-4), 72.2 (CH_2Ph), 71.0 (*C*-1), 70.5 (CH_2Ph) and 70.0 (CH_2Ph); HRMS *m/z* (ES^+) [Found: $(M+Na)^+$ 533.2159. $C_{29}H_{34}O_8SiNa$ requires M^+, 533.2151]; *m/z* (ES^+) 533 ($[M+Na]^+$, 100%). The data are in good agreement with the literature values.[28]

4.14. (−)-D-3-*O*-Benzyl-2,6-*O*-bisbenzyloxymethyl-*myo*-inositol 1,4,5-tris(dibenzyl phosphate) 14

To a solution of bis(benzyloxy)-*N*,*N*-bisisopropylamine phosphine (2.30 g, 6.65 mmol) in CH_2Cl_2 (100 mL) was added a solution of 1*H*-tetrazole (0.43 M in acetonitrile, 15.5 mL, 6.65 mmol). The mixture was stirred at rt for 20 min, before a solution of (+)-D-3-*O*-benzyl-2,6-*O*-bisbenzyloxymethyl-*myo*-inositol **13** (566 mg, 0.04 mmol) in CH_2Cl_2 (50 mL) was added dropwise via a pressure equalised dropping funnel over a period of 1 h. The resulting solution was stirred overnight at rt, after which time TLC analysis indicated the consumption of the starting material. The solution was cooled to −78 °C and 3-chloroperoxybenzoic acid (1.49 g, 6.65 mmol) was added in a single portion. The mixture was allowed to warm to rt and stirred for a further 3 h, after which TLC indicated consumption of the presumed intermediate (P^{III} species). The reaction was quenched by the addition of aqueous sodium sulfite solution (10% w/v, 100 mL). The layers were separated and the aqueous phase was re-extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layers were washed with brine (100 mL), dried over magnesium sulfate, filtered then concentrated in vacuo, providing a light brown oil. Purification of the oil by silica gel chromatography, eluting with hexane and ethyl acetate (50:50) provided (−)-D-3-*O*-benzyl-2,6-*O*-bisbenzyloxymethyl-*myo*-inositol 1,4,5-tris(dibenzyl phosphate) **14** (1.32 g, 93%) as a colourless oil: R_f 0.13 (hexane/ethyl acetate 1:1); $[\alpha]_D^{20} = -18.1$ (*c* 1.00, $CHCl_3$) {lit.[28] $[\alpha]_D^{24} = -17.6$ (*c* 0.85, $CHCl_3$)}; ^{1}H NMR (300 MHz; $CDCl_3$) δ 7.32–7.14 (45H, m, Ar *H*), 5.16–4.78 (17H, m, multiple CH_2), 4.71 (1H, d, *J* 12.1, CH_AH_BPh), 4.65 (2H, s, CH_2Ph), 4.56–4.48 (4H, m, 2 × ring *H* and CH_2), 4.43 (1H, dd, *J* 18.8, 9.4, ring *H*), 4.30 (1H, dd, *J* 9.5, 9.5, ring *H*), 4.17–4.10 (1H, m, *H*-5) and 3.37 (1H, dd, *J* 9.9, 2.1, *H*-3); ^{31}P NMR (121 MHz; $CDCl_3$) δ −1.12, −1.23 and −1.66; *m/z* (ES^+) 1313 ($[M+Na]^+$, 100%). The data are in good agreement with the literature values.[28]

4.15. (−)-D-*myo*-Inositol 1,4,5-trisphosphate hexakis sodium salt 15

To a solution of (−)-D-3-*O*-benzyl-2,6-*O*-bisbenzyloxymethyl-*myo*-inositol 1,4,5-tris(dibenzyl phosphate) **14** (120.0 mg, 0.093 mmol) in a mixture of *t*-butanol (6 mL) and water (1 mL) were added palladium black (198 mg, 1.859 mmol) and sodium hydrogen carbonate (46.8 mg, 0.558 mmol). The mixture was placed under vacuum and the atmosphere was replaced first with nitrogen gas and then with hydrogen gas. The suspension was then vigorously stirred under an atmosphere of hydrogen for 16 h. The heterogeneous mixture was filtered through a pad of Celite and the residue was washed with diethyl ether (2 × 5 mL); the organic filtrates were discarded. The palladium residue was washed with water (6 × 10 mL), and the combined aqueous filtrates were lypholised, providing (−)-D-*myo*-inositol 1,4,5-trisphosphate hexakis sodium salt **15** (40.5 mg, 95%) as a colourless solid:); $[\alpha]_D^{25} = -19.2$ (*c* 0.25, H_2O, pH ~9) {lit.[51] $[\alpha]_D^{24} = -20$ (*c* 0.05, H_2O, pH 9)}; ^{1}H NMR (300 MHz; D_2O) δ 4.21–4.15 (1H, m, *H*-2), 4.16–4.06 (1H, m, inositol ring), 3.92–3.75 (3H, m, inositol ring) and 3.59 (1H, dd, *J* 9.8, 2.7, *H*-1); ^{31}P NMR (121 MHz; D_2O) δ 3.82, 3.03 and 2.33; HRMS *m/z* (ES^+) [Found: $(M-Na+2H)^+$ 530.8791. $C_6H_{11}O_{15}P_3Na_5$ requires M^+, 530.8794]; *m/z* (ES^-) 485 ($[M-3Na+2H]^-$, 12%), 463 (40), 441 (63), 419 (52) and 339 (73). The data are in agreement with the literature values.[28] It should be noted that there is a large variation in the $[\alpha]_D$ values quoted for InsP_3 (−3.2 to −30) in the literature.

4.16. (−)-D-3-*O*-Benzyl-2,6-*O*-bisbenzyloxymethyl-5-*O*-(4-methoxybenzyl)-*myo*-inositol 16

To a solution of (−)-D-1-*O*-acetyl-3-*O*-benzyl-2,6-*O*-bisbenzyloxymethyl-5-*O*-(4-methoxybenzyl)-4-*O*-triisopropylsilyl-*myo*-inositol **11** (234 mg, 0.30 mmol) in THF (5 mL) was added a solution of tetrabutylammonium fluoride (1.0 M, 5.95 mL) in tetrahydrofuran. The solution was stirred at rt overnight, after which time TLC analysis showed consumption of the starting material. The reaction solution was diluted with water (20 mL), then extracted with diethyl ether (30 mL). The aqueous phase was re-extracted with CH_2Cl_2 (2 × 30 mL). The combined organic phases were washed with brine (20 mL), dried over magnesium sulfate, filtered and concentrated in vacuo, providing a colourless solid. Purification by silica gel chromatography, eluting with hexane and ethyl acetate (70:30, 50:50), provided *(−)-D-3-O-benzyl-2,6-O-bisbenzyloxymethyl-5-O-(4-methoxybenzyl)-myo-inositol* **16** (167 mg, 89%) as a colourless solid: R_f 0.13 (hexane/ethyl acetate 70:30); $[\alpha]_D^{20} = -16.9$ (*c* 0.54, $CHCl_3$); mp 105–106 °C (from hexane/ethyl acetate); ν_{max} (thin film)/cm^{-1} 3465 (s), 2892 (w), 1611 (m), 1514 (s), 1498 (m), 1454 (m), 1370 (m), 1251 (s), 1175 (s), 1065 (s), 1029 (s), 818 (w), 735 (s), 700 (m) and 586 (w); ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.38–7.26 (17H, m, Ar *H*), 6.91–6.87 (2H, m, *H*-3,5 $ArOCH_3$ ring), 5.00–4.97 (2H, m, overlapping 2 × CH_AH_B), 4.91 (1H, d, *J* 6.6, CH_AH_BPh), 4.85–4.58 (9H, m, multiple CH_AH_B), 4.28 (1H, dd, *J* 2.5, 2.5, *H*-2), 4.10 (1H, dd, *J* 9.7, 9.3, *H*-4), 3.85 (1H, dd, *J* 9.6, 9.3, *H*-6), 3.81 (3H, s, OCH_3), 3.78 (1H, d, *J* 4.7, O*H*-1), 3.29 (1H, ddd, *J* 9.6, 4.7, 2.4, *H*-1), 3.37 (1H, dd, *J* 9.3, 9.3, *H*-5), 3.31 (1H, dd, *J* 9.8, 2.5, *H*-3) and 2.55 (1H, br s, O*H*-4); ^{13}C NMR (100 MHz; $CDCl_3$) δ 159.2, 137.7, 137.6, 137.3, 130.9, 129.5, 128.6, 128.5, 128.4, 128.0, 128.0, 127.9, 127.7, 113.9 (*C*-3,5 $ArOCH_3$ ring), 96.7 (OCH_2O), 95.7 (OCH_2O'), 82.9 (*C*-6), 82.4 (*C*-5), 79.2 (*C*-3), 75.2 (*C*-2), 75.0 (CH_2), 73.0 (*C*-4), 72.1 (CH_2), 71.5 (*C*-1), 70.2 (CH_2), 69.7 (CH_2) and 55.3 (OCH_3); HRMS *m/z* (ES^+) [Found: $(M+Na)^+$ 653.2723. $C_{37}H_{42}O_9Na$ requires M^+, 653.2727]; *m/z* (ES^+) 653 ($[M+Na]^+$, 100%); Anal. Calcd for $C_{37}H_{42}O_9$: C, 70.46; H, 6.71. Found: C, 70.58; H, 6.62.

4.17. $^{45}Ca^{2+}$-flux assay in permeabilised Lvec and L15 fibroblasts

These experiments were performed as described in Kasri et al., 2004.[52] Briefly, Lvec cells were seeded in 12-well clusters (Greiner) at a density of 40,000 cells per well, whereas L15 cells were seeded at a density of 60,000 cells per well. Experiments were carried out on confluent cell monolayers between the 6th and 8th day after seeding. Cells were incubated for 10 min with a solution containing 120 mM KCl, 30 mM imidazole–HCl (pH 6.8), 2 mM $MgCl_2$, 1 mM

ATP, 1 mM EGTA and 40 μg/mL saponin at 30 °C. The non-mitochondrial Ca^{2+} stores were loaded for 45 min at 30 °C in 120 mM KCl, 30 mM imidazole–HCl (pH 6.8), 5 mM $MgCl_2$, 5 mM ATP, 0.44 mM EGTA, 10 mM NaN_3 and 150 nM of free Ca^{2+} (28 μCi/mL). The cells were then washed twice with 1 mL efflux medium containing 120 mM KCl, 30 mM imidazole–HCl (pH 6.8), 1 mM EGTA and 4 μM thapsigargin to block the ER Ca^{2+} pumps. The efflux medium was replaced every 2 min during 18 min and the Ca^{2+} content remaining in the stores was plotted as a function of time. After 10 min, cells were challenged with synthetic InsP_3, commercial InsP_3 or A23187 for 2 min. At the end of each experiment, the $^{45}Ca^{2+}$ remaining in the stores was released by incubation with 1 mL of a 2% SDS solution for 30 min. Commercial InsP_3 was obtained from Sigma.

4.18. MagFluo4 measurements in DT40 and TKO cells

These experiments were performed as described in Tovey et al., 2006.[53] Briefly, DT40 and TKO cells were grown to a density of about 1×10^6 cells per mL and loaded with Mag-Fluo4 AM (20 μM) for 60 min at 20 °C. Cells were permeabilised by incubation with saponin (10 μg/mL) for 4 min at 37 °C. After centrifugation, cells were resuspended in Mg^{2+}-free CLM (140 mM KCl, 20 mM NaCl, 1 mM EGTA and 20 mM PIPES (pH 7.0)), supplemented with 375 μM $CaCl_2$ and 10 μM FCCP, which inhibits mitochondrial Ca^{2+} uptake. Cells were dispensed into a 96-well black-walled assay plates pre-coated with poly-L-lysine and centrifuged to spin the permeabilised cells to the bottom of the wells. Cells were incubated with 90 μL of fresh Mg^{2+}-free CLM, supplemented with 375 μM $CaCl_2$ and 10 μM FCCP. MagFluo4 signals were measured with the FlexStation3 with automated additions (settings: excitation 490 nm, emission 525 nm, reading sensitivity normal (five readings) and photomultiplier tube (PMT) sensitivity high). At 30 s, 10 μL of Mg-ATP (15 mM, 1.5 mM final) is added to initiate Ca^{2+} uptake. After reaching steady-state store loading, 20 μL of thapsigargin (1 μM final) is added and 40 s later, 20 μL of ligands (synthetic InsP_3 or commercial InsP_3) was added at 7× the final concentration in medium containing 1 μM thapsigargin and 10 μM FCCP. Commercial InsP_3 was obtained from Sigma.

5. X-ray crystallographic data

Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 718377. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

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