Regioselective deprotection of orthobenzoates for the synthesis of inositol phosphates†

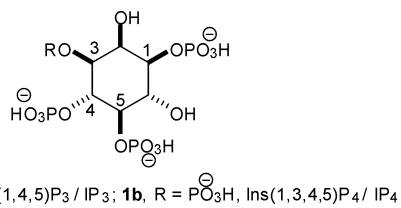
Joanna M. Swarbrick,[a] Samuel Cooper,[a] Geert Bultynck[b] and Piers R. J. Gaffney*[a]

Received 29th January 2009, Accepted 2nd February 2009
First published as an Advance Article on the web 6th March 2009
DOI: 10.1039/b901890e

Synthetic *myo*-inositol 1,4,5-triphosphate, Ins(1,4,5)P_3, and *myo*-inositol 1,3,4,5-tetraphosphate, Ins(1,3,4,5)P_4, continue to be valuable in biological studies. Inositol orthoesters have proved an important class of intermediate to access these compounds. We investigated the ability of steric bulk from a 4-*O* protecting group to direct DIBAL-H reduction of inositol orthobenzoates to generate the natural Ins(1,4,5)P_3 precursor 2,3,6-*O*-tribenzyl *myo*-inositol. Introduction of an equatorial 4-*C*-methyl group imparts totally selective reduction and we report the synthesis of novel 4-*C*-methyl-Ins(1,4,5)P_3 and 4-*C*-methyl-Ins(1,3,4,5)P_4.

Introduction

The inositol phosphates, particularly inositol 1,4,5-tri- and 1,3,4,5-tetraphosphates (Fig. 1, **1a** and **1b** respectively), are best known as cytosolic secondary messengers.[1] They can be phosphorylated on all six hydroxyls, with most possible regioisomers from inositol mono- to hexa-phosphate known in nature, and are interconverted by a battery of kinases and phosphatases. Disentanglement of this signalling network is difficult due to the often interdependent mechanisms of action, and the number of different pathways in which they are involved. Identification of the participation of these species in signalling pathways, perturbation of which leads to disease states such as cancer and diabetes, has further increased the need to understand this complex system. Inositol phosphates are neither readily synthesised, nor isolable from nature, leading to a wealth of multi-step synthetic routes.[2] Use of unnatural analogues as biological probes should help elucidate information about how they function.

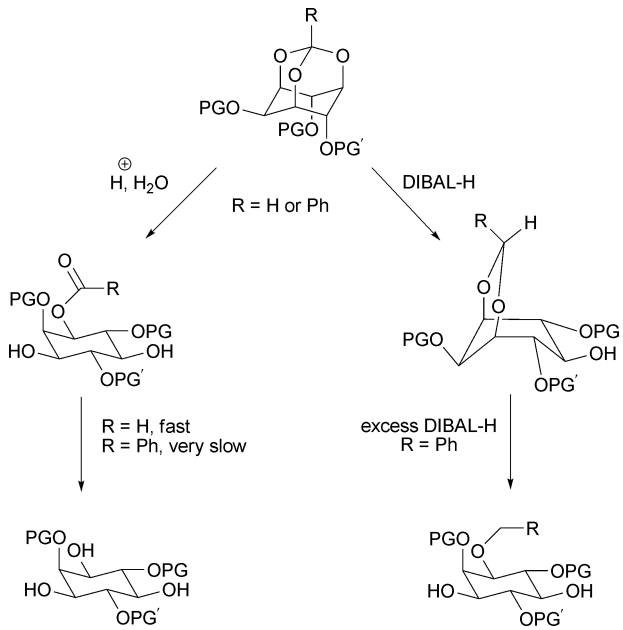


1a, R = H, Ins(1,4,5)P_3 / IP_3; **1b**, R = PO_3H, Ins(1,3,4,5)P_4 / IP_4

Fig. 1 Inositol 1,4,5-triphosphate and inositol 1,3,4,5-tetraphosphate.

Inositol phosphate synthesis requires a comprehensive protecting group strategy, often commencing from readily prepared building blocks such as inositol acetals or orthoesters. The principal advantages of orthoesters lie in their simultaneous protection of the 1-, 3- and 5-hydroxyls, restricting the *myo*-inositol conformation, and subsequent easy differentiation between the remaining three hydroxyls.[3] Past synthetic focus has been on the orthoformates.[4,5] However, trans-esterification of *myo*-inositol with triethyl orthobenzoate generates *myo*-inositol orthobenzoate (**2**) which is easily purified by recrystallisation in good yield.[6]

Full or partial deprotection of orthoesters can be carried out by either acidic hydrolysis or reduction. Manipulation of this deprotection has the potential to generate further protecting groups and selectively expose hydroxyl functionalities (Scheme 1).



Scheme 1 Deprotection of orthoesters.

Acidic hydrolysis of an inositol orthoformate presumably generates a formate ester initially, which is rapidly hydrolysed to reveal all three hydroxyls.[7] By contrast acidic hydrolysis of orthobenzoates generates a 1(3)-*O*-benzoate ester (Bz), which can

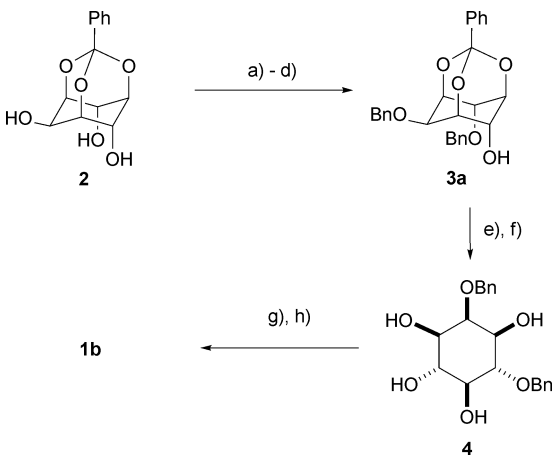
[a]*Department of Chemistry, Imperial College London, South Kensington Campus, SW7 2AZ, UK. E-mail: p.gaffney@imperial.ac.uk; Fax: +44 (020) 7594 5804; Tel: +44 (020) 7594 5881*
[b]*Department of Molecular Cell Biology, Laboratory of Molecular and Cellular Signaling, Campus Gasthuisberg o/n-1 bus 802, Herestraat 49, BE-3000, Leuven, Belgium. E-mail: geert.bultynck@med.kuleuven.be; Fax: +32 (16) 330215; Tel: +32 (16) 345991*

† Electronic supplementary information (ESI) available: Preparation of compounds **2–3d** and **8–10**, plus all analytical data. See DOI: 10.1039/b901890e

be isolated. Alternatively reduction of the orthoformate with 2 eq. DIBAL-H generates a bridging 1,3-*O*-methylidene acetal;[8] this is a much less acid labile protecting group than the benzylidene acetal derived from reduction of an orthobenzoate.[9] Additionally, further reduction of the benzylidene acetal with excess DIBAL-H produces a benzyl ether (Bn). If PG ≠ PG′ both the 1- and 3-*O*-benzyl isomers result from reduction of the asymmetric benzylidene acetal. Thus, if the easily prepared 2,6-*O*-dibenzyl orthobenzoate **3a** can be similarly reduced with DIBAL-H, the resultant 2,3,6-*O*-tribenzyl ether **6a** is the synthetically useful precursor to inositol 1,4,5-triphosphate (**1a**). Consequently, we sought to affect the regioselectivity of DIBAL-H reduction of **3a** to favour isomer **6a** over the unwanted 1,2,6-*O*-tribenzyl ether **7a**.

Results and discussion

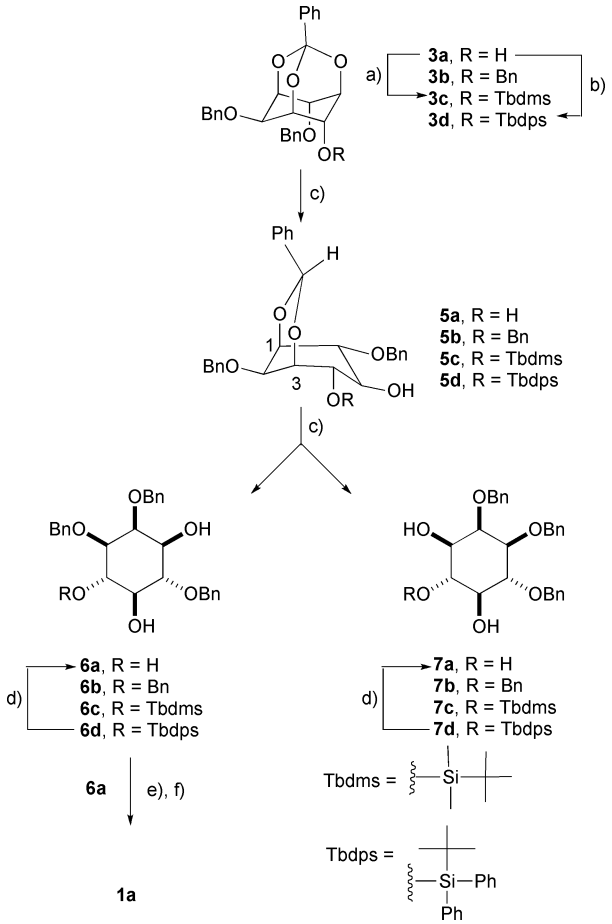
IP_4 (**1b**) may be synthesised from readily prepared dibenzyl ether **3a** (Scheme 2). Key steps were direct regioselective allylation of crude inositol orthobenzoate to give the 4-*O*-allyl ether in good yield (73%).[10] This directed benzylation of the 2,6-diol to provide the established IP_4 building block **4**, after unblocking of the temporary protecting groups.[6,11] Phosphorylation and global deprotection proceeded as previously reported.[12a] Intermediate **3a** offers potential for resolution, for example with camphanate esters,[13] and was the key starting point for our investigations of orthobenzoate hydrolysis and reduction.



Scheme 2 Synthesis of IP_4. *Reagents and conditions*: a) NaH, allylBr; b) NaH, BnBr, 60 °C; c) ^{t}BuOK; d) TsOH; e) TFA-H_2O (4:1), 24 h; f) NaOMe, MeOH, 75 °C; g) $(BnO)_2PNiPr_2$, tetrazole, then *m*CPBA; h) Pd (black), H_2.

Partial reduction of 2,4,6-*O*-tribenzyl ether **3b**, synthesised by exhaustive benzylation of inositol orthobenzoate (**2**), with limited DIBAL-H is known to give **5b** in good yield.[9] However, upon addition of 3.5 eq. DIBAL-H to **3b** we observed total reduction of the orthobenzoate to 1,2,4,6-*O*-tetrabenzyl inositol (**6b** ≡ **7b**, Scheme 3) generated by a second hydride insertion into the 1(3)-O-C bond of symmetrical 1,3-*O*-benzylidene acetal **5b**. We then explored the full reduction of **3a** with DIBAL-H in the hope of selectively producing the building block **6a** required for IP_3 (**1a**) synthesis.

Treatment of asymmetrical *myo*-inositol orthobenzoate **3a** with 3.5 eq. DIBAL-H generates both 2,3,6-*O*-tribenzyl-*myo*-inositol



Scheme 3 Reduction of *myo*-inositol orthobenzoates. *Reagents and conditions*: a) TbdmsCl, imidazole, Et_3N, DMF, 100 °C; b) TbdpsCl, imidazole, Et_3N, DMF, 100 °C; c) DIBAL-H, DCM, −78 °C to rt; d) TBAF, THF; e) $(BnO)_2PN^iPr_2$, tetrazole then *m*CPBA; f) Pd (black), H_2.

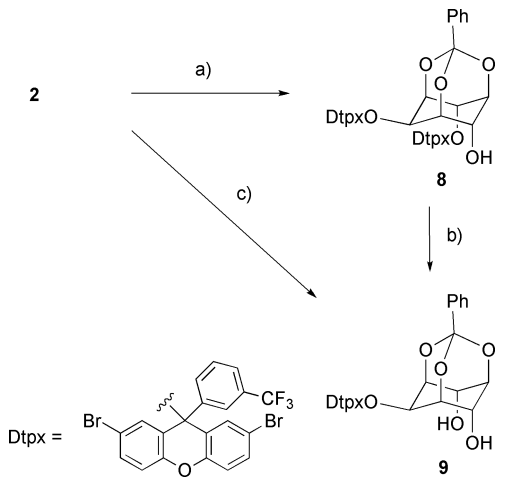
6a[12b] and 1,2,6-*O*-tribenzyl-*myo*-inositol **7a**, which are easily separated by flash chromatography. However, the highly polar triols were recovered in poor yield due to difficulty in handling and there was little selectivity for the desired isomer **6a** as determined by ^{1}H-NMR of the crude product mixture (Table 1). To complete the synthesis of IP_3 (**1a**), **6a** was phosphorylated and globally deprotected as previously described.[12a]

We had anticipated that slow addition of DIBAL-H to **3a** would favour reduction to **6a** due to initial rapid reaction of the unprotected 4-*O* with bulky DIBAL-H. This was thought to occur readily because treatment of **8** with 2 eq. DIBAL-H exclusively unblocked the acid labile 6-*O*-Dtpx ether to give symmetrical mono 2-*O*-Dtpx ether **9**, presumably by trans-annular

Table 1 Ratio of crude products in ^{1}H-NMR of DIBAL-H reduction of **3**

		Ratio of products		
Starting material	R	3-*O*-Bn, **6**	:	1-*O*-Bn, **7**
3a	H	4	:	3
3b	Bn	1	:	1
3c	Tbdms	4	:	5
3d	Tbdps	4	:	5

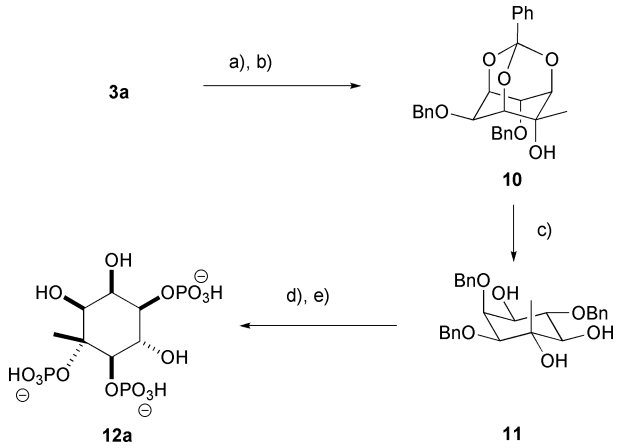
chelation to the 6-*O* of the Lewis acidic 4-*O*-DIBAL adduct (Scheme 4). The identity of **9** was confirmed by alkylating **2** with DtpxCl under milder conditions than those required to give **8**; this reaction is notable for its unusual regioselectivity for the 2-*O* which has previously been observed with a few large electrophiles under mildly basic conditions.[4,7,14] Unfortunately variation of the temperature and speed of addition of DIBAL-H to **3a** had little effect on the **6a**:**7a** ratio.



Scheme 4 Selective 4-*O*-Dtpx unblocking with DIBAL-H. *Reagents and conditions*: a) DtpxCl, MeCN, pyridine, reflux; b) DIBAL-H, DCM, 0 °C; c) DtpxCl, pyridine.

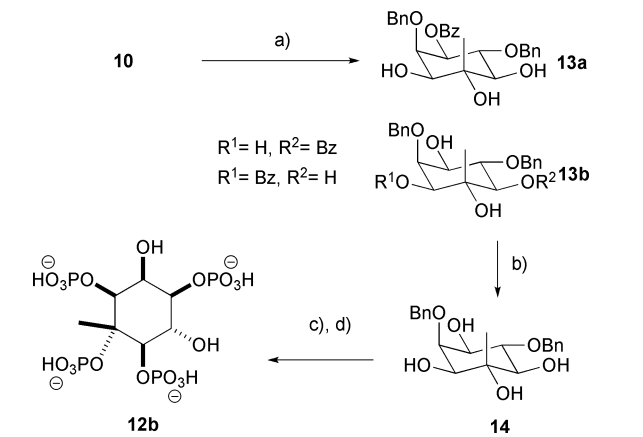
We attempted to affect the regioselectivity of the second DIBAL-H reaction by increasing the steric congestion at the 4-*O* with a bulky silyl ether. From its ^{1}H-NMR the inositol ring of benzylidene **5b** is known to occupy a boat conformation [$\delta_{5\text{-H}}$ = 3.79 (t, *J* 8.6)][9] and it was anticipated that restricted access of DIBAL-H to the 3-*O* in benzylidene intermediates **5c** and **5d** would favour reduction of the 1-*O* acetal bond. Therefore 4-*O*-*tert*-butyldimethylsilyl (Tbdms) ether **3c** and the bulkier 4-*O*-*tert*-butyldiphenylsilyl (Tbdps) derivative **3d** were prepared by silylation of **3a** under forcing conditions. The resultant silyl ethers were treated with excess DIBAL-H, but in neither case was one regioisomer preferred, although the reduction products were easier to handle and separate than **6a** and **7a**. The identities of **6d** and **7d** were confirmed by desilylation with TBAF to give **6a** and **7a** respectively. This lack of selectivity may be because the substrates for the second reduction of **3c** and **3d** with excess DIBAL-H are not benzylidenes **5c** and **5d** but rather their 5-*O*-DIBAL-adducts. The protection of *trans*-cyclohexane 1,2-diol with bulky silyl ethers is known to favour the 1,2-diaxial chair conformation[15] and repulsion between the bulky 4-*O*-silyl and 5-*O*-DIBAL groups may force the inositol ring of **5c** and **5d** back into a chair-like conformation with 4,5,6-triaxial substituents reducing the contrast between the steric environment at the 1- and 3-*O*.

Noting that the equatorial 2-*O*-Bn limits access to the 1- and 3-*O* of **3**, we postulated that access to the 3-*O* would be further constrained by a second adjacent equatorial substituent. 4-*C*-Methyl inositol orthobenzoate **10** was prepared from **3a** by oxidation of the 4-OH to the inos-4-ose, followed by stereoselective equatorial addition of a methyl group to re-establish the *myo*-inositol stereochemistry[16] (Scheme 5). Treatment of **10** with 4 eq. DIBAL-H then generated exclusively the IP_3 analogue precursor **11** and there was no evidence of the potential 1-*O*-benzyl regioisomer. Phosphitylation of the three free hydroxyls, including the unnatural tertiary centre, followed by oxidation and then global deprotection of the nine benzyl groups generated the novel IP_3 analogue **12a**.



Scheme 5 Synthesis of 4-*C*-Me IP_3. *Reagents and conditions*: a) Dess–Martin periodinane, DCM; b) MeMgBr, ether, −78 °C to rt, 3 h; c) 4 eq. DIBAL-H, DCM, −78 °C to rt, 4 h; d) $(BnO)_2PN^iPr_2$, tetrazole followed by *m*CPBA; e) Pd (black), H_2.

The 4-*C*-methyl IP_4 analogue, **12b**, was also synthesised (Scheme 6). Previously reported conditions for orthobenzoate removal [TFA-water (4:1), as with **3a**], were not sufficient to initiate acid hydrolysis of **10**. Treatment of **10** with conc. HCl-MeOH at reflux generated a mixture of benzoate esters **13a** and **13b**. Similarly to hydrolysis of orthobenzoate **3a**,[17] there was no preference for the 1-*O* benzoate. However, as has been described for **3a**, the 1-*O*-benzoate may be isolated and used in the preparation of the corresponding 1-*O*-phosphatidyl inositol triphosphate. The same is assumed for the 4-*C*-methyl lipid head group precursor **13a**. The marked increase in orthobenzoate stability compared to **3a** may be interpreted in terms of the established mechanism for *cis*,*cis*-cyclohexane 1,3,5-triol orthoester hydrolysis[18] where the rate determining step is the chair-boat interconversion of the inositol ring of the intermediate cation to permit irreversible



Scheme 6 Synthesis of 4-*C*-Me IP_4. *Reagents and conditions*: a) conc. HCl-MeOH (1:2), 70 °C, 3 h; b) NaOMe, MeOH, 75 °C, 3 h; c) $(BnO)_2PN^iPr_2$, tetrazole then *m*CPBA; d) Pd (black), H_2.

trapping of the dioxacarbenium ion by water. It is postulated that the additional equatorial substituent on the inositol ring of 4-*C*-methyl **10** further hinders chair-boat flipping of the bicyclic cation. The mixed benzoyl esters **13a** and **13b** were treated with sodium methoxide in methanol to give **14**, the tetraacetate derivative of which exhibited a clear NOESY cross-peak between the 4-*C*-methyl singlet and the inositol ring 6-H, consistent with the expected 4-*C* stereoisomer having the methyl group axial.[16] Tetrol **14** was subsequently phosphorylated and deprotected (as described for **11**) to generate IP_4 analogue **12b**.

Both **12a** and **12b** occupy the chair conformation, as indicated by the large coupling constants between the three ring protons having an anti relationship. For example the coupling constants between the anti-periplanar 5- and 6-*H* (for **12a**, $J_{5,6}$ 9.6; for **12b**, $J_{5,6}$ 9.7) are very similar to that in the natural ligands (for **1a**, $J_{5,6}$ 9.2; for **1b**, $J_{5,6}$ 9.4); this would have been reduced in a boat-like conformation with the 4-*C*-methyl occupying a pseudo-equatorial position. The similarity of conformation between natural IP_3 **1a** and 4-*C*-methyl analogue **12a** was reflected by the latter's ability to open the IP_3 receptor (IP_3R) Ca^{2+}-release channels in permeabilised L15 cells (Fig. 2).[19,20] IP_3 (**1a**) was found to release *ca.* 70% of available Ca^{2+} (by comparison with that released by the Ca^{2+} ionophore A23187) with an EC_{50} of 1.1 μM, whereas racemic 4-*C*-methyl IP_3 (**12a**) released *ca.* 50% with a lower EC_{50} of 13.8 μM.

Conclusions

The utility of orthobenzoate protection of inositol is demonstrated in the synthesis of inositol phosphates **1a** and **1b**, by DIBAL-H reduction and acidic hydrolysis of a common intermediate. Introduction of a methyl substituent on the 4-*C* significantly reduces the rate of acidic hydrolysis but enforces total regiocontrol during DIBAL-H reduction. This allows facile access to the novel 4-*C*-methyl derivatives of biologically active Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4, **12a** and **12b**.

Experimental

General experimental

^{1}H-, ^{13}C- and ^{31}P-NMR spectra were recorded on Bruker AC-270, AM-360, AV-400 and AV-500 spectrometers. Chemical shifts are referenced with respect to residual solvent signals, δ_H ($CHCl_3$) 7.25 ppm, δ_H (d_5-DMSO) 2.50 ppm, δ_H (HOD) 4.60 ppm, δ_C ($CDCl_3$) 77.50 ppm, δ_C (d_6-DMSO) 39.43 ppm, or an external reference, δ_P (H_3PO_4) 0.00 ppm. The splitting patterns for ^{1}H-NMR spectra are denoted as follows; s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), b (broad) and combinations thereof. Coupling constants (*J*) are in Hertz (Hz). ^{13}C-NMR assignments and ^{1}H-NMR assignments were made with the aid of DEPT-90 and -135, HSQC, COSY and NOESY experiments. ^{13}C- and ^{31}P-NMR are proton decoupled. Deuterated solvents were purchased from *Apollo Scientific Ltd* (d_6-DMSO) or *Merck* (all others). Other reagents were purchased from *Sigma-Aldrich Ltd* or *Acros Organics* and used as supplied except where specified. Mass spectra were recorded on a *VG* AutoSpec-Q (CI) or a *Micromass* LCT Premier (ESI) mass spectrometer. Reactions were carried out under anhydrous conditions under a nitrogen atmosphere. Dichloromethane, acetonitrile, toluene and triethylamine were distilled from calcium hydride; THF and diethyl ether were distilled from sodium metal and benzophenone; methanol was distilled from magnesium methoxide; all, except triethylamine, were stored over 4 Å molecular sieves. Flash chromatography was carried out using flash silica and medium pressure liquid chromatography using TLC grade silica from *Merck*. Thin layer chromatography was carried out using *Merck* silica gel 60 F_{254} glass-backed plates, compounds were visualised using UV light or $KMnO_4$ stain.

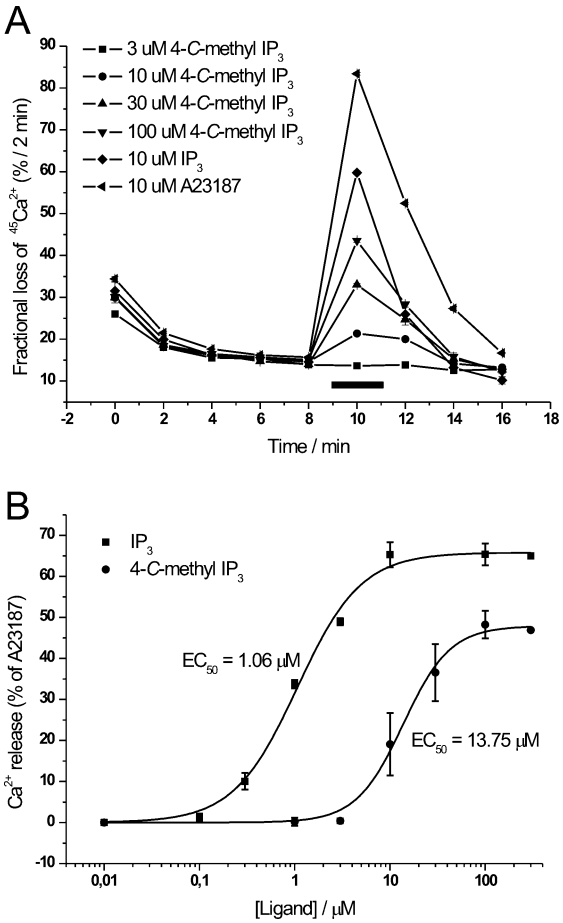


Fig. 2 (A) A typical experiment in permeabilised L15 cells showing the fractional loss of $^{45}Ca^{2+}$ with time. The black bar indicates the addition of IP_3 (**1a**) or 4-*C*-methyl IP_3 (**12a**). Cells were treated with A23187 to estimate the maximal releasable $^{45}Ca^{2+}$. (B) Dose response for IP_3R-dependent Ca^{2+} release provoked by IP_3 (**1a**) or 4-*C*-methyl IP_3 (**12a**) in permeabilised L15 cells. Values were normalized to the A23187-releasable Ca^{2+}. Data points were obtained from at least 3 independent experiments and are plotted as mean ± standard error of the mean. EC_{50} values were obtained by sigmoidal curve fitting (Origin® 7.0).

General method for the total reduction of *myo*-inositol orthobenzoates with DIBAL-H

The *myo*-inositol 1,3,5-*O*-orthobenzoate (**3a-d**, 0.50 mmol) was evaporated from toluene (3 × 2 mL), taken up in CH_2Cl_2 (5 mL) and cooled to −78 °C. DIBAL-H (0.7 M in hexanes, 3.5 eq.) was added dropwise (initial vigorous effervescence observed), the solution warmed to rt over 3 h and stirred for a further 12 h.

The reaction was quenched with H_2O (25 mL) and extracted with CH_2Cl_2 (4 × 100 mL). The combined organic layers were washed with H_2O (50 mL) and brine (50 mL), dried ($MgSO_4$), filtered and evaporated to dryness under reduced pressure.

2,3,6-*O*-Tribenzyl-*myo*-inositol (6a) and 1,2,6-*O*-tribenzyl-*myo*-inositol (7a). 2,6-*O*-Dibenzyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (**3b**, 225 mg, 0.50 mmol) was reduced with DIBAL-H using the general method described above. The residue was fractionated by chromatography on flash silica. Elution with hexane-EtOAc (9:1 → 2:8 v/v) afforded **6a** (60 mg, 26%) and **7a** (40 mg, 18%) both as off-white solids; for **6a** R_f (hexane-EtOAc, 3:7 v/v) 0.46; δ_H (400 MHz, d_6-DMSO) 7.42–7.24 (15H, m, 15 × Ar C*H*), 4.96 (1H, d, *J* 5.1, ex, Ins 1-O*H*), 4.93 (1H, d, *J* 4.9, ex, Ins 4-O*H*), 4.91 (1H, d, *J* 5.2, ex, Ins 5-O*H*), 4.83 (1H, d, *J* 11.9, OCH*H*Ph), 4.81 (1H, d, *J* 11.5, OCH*H*Ph), 4.76 (1H, d, *J* 11.5, OCH*H*Ph), 4.73 (1H, d, *J* 11.9, OCH*H*Ph), 4.64 (2H, s, OCH_2Ph), 3.96 (1H, bs, Ins 2-*H*), 3.64 (1H, td, *J* 9.4, 4.9, ex → t, Ins 4-*H*), 3.51–3.44 (2H, m, Ins 5-*H* + Ins 1-*H*), 3.25 (1H, dd, *J* 9.9, 2.3, Ins 3-*H*), 3.18 (1H, td, *J* 8.8, 5.2, ex → t, Ins 6-*H*) ppm [the ^{1}H-NMR in $CDCl_3$ (not given) is consistent with lit.,[12b] which is the best resolved published data we are aware of, but is incomplete lacking two of the inositol ring resonances]; δ_C (100 MHz, d_6-DMSO) 140.23, 140.06, 139.59 (3 × Ar *C*), 128.57 (2C), 128.45 (2C), 128.33 (2C), 128.03, 127.84 (2C), 127.67 (4C), 127.53, 127.43 (15 × Ar *C*H), 82.45, 80.56, 79.42, 75.60 (4 × Ins *C*H), 74.51, 74.10 (2 × OCH_2Ph), 73.17, 71.97 (2 × Ins *C*H), 71.91 (OCH_2Ph) ppm; HRMS (CI+) *m*/*z* (%) found $[M+Na]^+$ 473.1947 (100), $C_{27}H_{30}O_6Na$ requires 473.1940: For **7a** R_f (hexane-EtOAc, 3:7 v/v) 0.18; mp 136–137.5 °C; δ_H (500 MHz, d_6-DMSO) 7.31–7.21 (15H, m, 15 × Ar *H*), 4.88 (1H, d, *J* 5.2, ex, Ins 5-O*H*), 4.81–4.70 [6H, m, Ins 3-O*H* (ex) + Ins 4-O*H* (ex) + (2 × OCH_2Ph)], 4.62 (1H, d, *J* 11.9, OCH*H*Ph), 4.54 (1H, d, *J* 11.9, OCH*H*Ph), 3.98 (1H, t, *J* 2.4, Ins 2-*H*), 3.58 (1H, t, *J* 9.5, Ins 6-*H*), 3.47 (1H, td, *J* 9.5, 4.6, ex → t, Ins 4-*H*), 3.45 (1H, dd, *J* 9.9, 2.5, Ins 1-*H*), 3.27 (1H, ddd, *J* 9.8, 4.6, 2.4, ex → dd, Ins 3-*H*), 3.16 (1H, td, *J* 9.0, 5.2, ex → t, Ins 5-*H*) ppm; δ_C (125 MHz, $CDCl_3$) 138.64 (2C), 138.10 (3 × Ar *C*), 128.48 (4C), 128.43 (2C), 128.07 (2C), 127.81 (2C), 127.78 (2C), 127.73, 127.64 (2C) (15 × Ar *C*H), 81.11, 80.92, 77.34 (3 × Ins *C*H), 75.48, 74.89 (2 × OCH_2Ph), 74.51, 73.60 (2 × Ins *C*H), 72.85 (OCH_2Ph), 72.10 (Ins *C*H) ppm; HRMS (CI+) *m*/*z* (%) found $[M+Na]^+$ 473.1947 (100), $C_{27}H_{30}O_6Na$ requires 473.1940.

1,2,4,6-*O*-Tetrabenzyl-*myo*-inositol (6b ≡ 7b). 2,4,6-*O*-Tribenzyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (**3a**, 100 mg, 0.19 mmol) was reduced with DIBAL-H using the general method described above, affording **6b** (102 mg, 100%) as a clear oil; R_f (hexane-EtOAc, 1:1 v/v) 0.48; δ_H (400 MHz, $CDCl_3$) 7.41–7.29 (20H, m, 20 × Ar *H*), 5.03 (1H, d, *J* 11.6, OC*H*HPh), 5.02 (1H, d, *J* 11.2, OC*H*HPh), 4.93 (1H, d, *J* 11.3, OC*H*HPh), 4.82 (1H, d, *J* 11.4, OC*H*HPh), 4.80 (1H, d, *J* 11.2, OC*H*HPh), 4.77 (1H, d, *J* 11.6, OC*H*HPh), 4.71 (2H, s, OCH_2Ph), 4.09 (1H, t, *J* 2.5, Ins 2-*H*), 3.95 (1H, t, *J* 9.4, Ins 4-*H*), 3.74 (1H, t, *J* 9.3, Ins 6-*H*), 3.58 (1H, t, *J* 9.1, Ins 5-*H*), 3.52 (1H, bd, *J* 10.1, Ins 1-*H*), 3.48 (1H, dd, *J* 9.7, 2.4, Ins 3-*H*), 2.57 (1H, bs, Ins 5-O*H*), 2.38 (1H, bs, Ins 1-O*H*) ppm; δ_C (100 MHz, $CDCl_3$) 138.66, 138.62 (2C), 138.05 (4 × Ar *C*), 128.50 (4C), 128.45 (2C), 128.37 (2C), 128.03 (2C), 128.00 (2C), 127.77 (4C), 127.75, 127.65, 127.60 (2C) (20 × Ar *C*H), 81.66 (Ins 6-*C*H), 81.25 (Ins 4-*C*H), 80.87 (Ins 3-*C*H), 77.05 (Ins 2-*C*H), 75.48 (OCH_2Ph), 74.98 (Ins 5-*C*H), 74.98, 74.94, 72.70 (3 × OCH_2Ph), 72.18 (Ins 1-*C*H) ppm; MS (CI+) *m*/*z* (%) $[M+H]^+$ 540 (100).

2,3,6-*O*-Tribenzyl-4-*O*-*tert*-butyldimethylsilyl-*myo*-inositol (6c) and 1,2,6-*O*-tribenzyl-4-*O*-*tert*-butyldimethylsilyl-*myo*-inositol (7c). 2,6-*O*-Dibenzyl-4-*O*-*tert*-butyldimethylsilyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (**3c**, 100 mg, 0.15 mmol) was reduced by DIBAL-H using the general method described above. The residue (86 mg) was fractionated by chromatography on flash silica. Elution with hexane-EtOAc (9:1 → 3:7 v/v) afforded **6c** (42 mg, 42%) and **7c** (40 mg, 40%) both as pale yellow oils; for **6c** R_f (hexane-EtOAc, 1:1 v/v) 0.63; δ_H (400 MHz, $CDCl_3$) 7.41–7.30 (15H, m, 15 × Ar *H*), 4.94 (1H, d, *J* 11.6, 6-OC*H*HPh), 4.91 (1H, d, *J* 11.5, 2-OC*H*HPh), 4.83 (1H, d, *J* 11.2, 2-OC*H*HPh), 4.69 (1H, d, *J* 11.5, 6-OC*H*HPh), 4.66 (2H, s, 3-OCH_2Ph), 4.04 (1H, t, *J* 9.1, Ins 4-*H*), 4.02 (1H, t, *J* 2.8, Ins 2-*H*), 3.68 (1H, t, *J* 9.3, Ins 6-*H*), 3.53 (1H, ddd, *J* 9.2, 6.4, 2.6, ex → dd, Ins 1-*H*), 3.45 (1H, td, *J* 8.9, 2.1, ex → t, Ins 5-*H*), 3.27 (1H, dd, *J* 9.5, 2.2, Ins 3-*H*), 2.44 (1H, d, *J* 2.2, Ins 5-O*H*), 2.33 (1H, d, *J* 6.6, Ins 1-O*H*), 0.92 (9H, s, SiCMe_3), 0.17 (3H, s, Si*Me*), 0.09 (3H, s, Si*Me*) ppm; δ_C (125 MHz, $CDCl_3$) 138.79, 138.70, 137.99 (3 × Ar *C*), 128.47 (2C), 128.35 (3C), 127.98 (2C), 127.73, 127.62 (7C) (15 × Ar *C*H), 81.77, 80.91, 77.11, 76.09 (4 × Ins *C*H), 74.96, 74.79 (2 × OCH_2Ph), 74.07 (Ins *C*H), 72.73 (OCH_2Ph), 72.32 (Ins *C*H), 25.99 (SiCMe_3), 18.31 (Si*C*Me$_3$), –4.06, –4.55 (2 × Si*Me*) ppm; HRMS (ESI+) *m*/*z* (%) found $[M+H]^+$ 565.2972 (100), $C_{33}H_{45}O_6Si$ requires 565.2985: for **7c** R_f (hexane-EtOAc, 1:1 v/v) 0.72; δ_H (400 MHz, $CDCl_3$) 7.41–7.31 (15H, m, 15 × Ar *H*), 5.02 (1H, d, *J* 12.1, 2-OC*H*HPh), 4.99 (1H, d, *J* 11.9, 6-OC*H*HPh), 4.78 (1H, d, *J* 11.4, 6-OC*H*HPh), 4.75 (1H, d, *J* 12.1, 2-OC*H*HPh), 4.70 (2H, s, 1-OCH_2Ph), 4.06 (1H, t, *J* 2.6, Ins 2-*H*), 3.90 (1H, t, *J* 9.5, Ins 6-*H*), 3.80 (1H, t, *J* 9.1, Ins 4-*H*), 3.48 (1H, dd, *J* 9.7, 2.4, Ins 1-*H*), 3.37 (1H, t, *J* 9.0, Ins 5-*H*), 3.34 (1H, dd, *J* 9.4, 2.6, Ins 3-*H*), 2.43 (1H, bs, Ins O*H*), 2.17 (1H, bs, Ins O*H*), 0.93 (9H, s, SiCMe_3), 0.14 (3H, s, Si*Me*), 0.13 (3H, s, Si*Me*) ppm; δ_C (100 MHz, $CDCl_3$) 128.72 (2C), 138.7 (3 × Ar *C*), 128.41 (2C), 128.38 (3C), 127.89 (2C), 127.70, 127.66 (2C), 127.64 (3C), 127.61 (2C) (15 × Ar *C*H), 81.14, 80.82, 77.09, 75.47 (4 × Ins *C*H), 75.47 (OCH_2Ph), 75.23 (Ins *C*H), 74.66 (OCH_2Ph), 72.95 (Ins *C*H), 72.70 (OCH_2Ph), 25.93 (SiCMe_3), 18.30 (Si*C*Me$_3$), –4.28, –4.44 (2 × Si*Me*) ppm; HRMS (ESI+) *m*/*z* (%) found $[M+H]^+$ 565.2992 (100), $C_{33}H_{45}O_6Si$ requires 565.2985.

2,3,6-*O*-Tribenzyl-4-*O*-*tert*-butyldiphenylsilyl-*myo*-inositol (6d) and 1,2,6-*O*-tribenzyl-4-*O*-*tert*-butyldiphenylsilyl-*myo*-inositol (7d). 2,6-*O*-Dibenzyl-4-*O*-*tert*-butyldiphenylsilyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (**3d**, 460 mg, 1.07 mmol) was reduced with DIBAL-H using the general method described above. The residue (430 mg) was fractionated by chromatography on flash silica. Elution with hexane-EtOAc (9:1 → 7:3 v/v) afforded **6d** (153 mg, 33%) and **7d** (193 mg, 42%) both as clear oils; for **6d** R_f (hexane-EtOAc, 7:3 v/v) 0.34; δ_H (400 MHz, $CDCl_3$) 7.76–7.68 (4H, m), 7.45–7.18 (17H, m), 7.14–7.12 (2H, m), 6.96–6.94 (2H, m) (25 × Ar *H*), 4.88 (1H, d, *J* 11.2, 6-OC*H*HPh), 4.71 (1H, d, *J* 11.2, 6-OC*H*HPh), 4.59 (1H, d, *J* 11.5, 2-OC*H*HPh), 4.53 (1H, d, *J* 11.4, 2-OC*H*HPh), 4.41 (1H, d, *J* 11.3, 3-OC*H*HPh), 4.27 (1H, t, *J* 8.7, Ins 4-*H*), 4.19 (1H, d, *J* 11.3, 3-OC*H*HPh), 4.04 (1H, t, *J* 2.1, Ins 2-*H*), 3.70 (1H, td, *J* 8.0, 2.7, Ins 5-*H*), 3.67–3.60 (2H, m, Ins 1-*H* + Ins 6-*H*), 3.41 (1H, dd, *J* 9.0, 2.1, Ins 3-*H*), 2.41 (1H, d, *J* 3.0, Ins 5-O*H*), 2.35 (1H, d, *J* 4.3, Ins 1-O*H*), 1.04 (9H, s,

SiC*Me*$_3$) ppm; δ_C (100 MHz, $CDCl_3$) 138.69, 138.65, 137.62 (3 × Ar *C*), 135.94 (2C), 135.87 (2C) (4 × Ar *C*H), 133.88, 133.77 (2 × Ar *C*), 129.48, 129.44, 128.42 (2C), 128.18 (2C), 128.02 (2C), 127.93 (2C), 127.70 (2C), 127.56 (2C), 127.45 (2C), 127.36 (5C) (21 × Ar *C*H), 81.60, 80.95, 76.29, 75.83, 75.03 (5 × Ins *C*H), 74.88, 74.11 (2 × O*C*H_2Ph), 72.23 (Ins *C*H), 71.77 (O*C*H_2Ph), 27.06 (SiC*Me*$_3$), 19.65 (Si*C*Me_3) ppm; HRMS (ESI+) *m*/*z* (%) found $[M+Na]^+$ 711.3102 (100), $C_{43}H_{48}O_6SiNa$ requires 711.3118: for **7d** R_f (hexane-EtOAc, 7:3 v/v) 0.62; δ_H (400 MHz, $CDCl_3$) 7.75–7.72 (4H, m), 7.44–7.26 (19H, m), 7.07–7.05 (2H, m) (25 × Ar *H*), 4.88 (2H, d, *J* 11.3, 2-OC*H*HPh + 6-OC*H*HPh), 4.75 (1H, d, *J* 10.9, 6-OC*H*HPh), 4.69 (1H, d, *J* 11.9, 1-OC*H*HPh), 4.66 (1H, d, *J* 11.8, 1-OC*H*HPh), 4.49 (1H, d, *J* 11.8, 2-OC*H*HPh), 3.90 (1H, t, *J* 2.6, Ins 2-*H*), 3.89 (1H, t, *J* 8.9, Ins 4-*H*), 3.77 (1H, t, *J* 9.3, Ins 6-*H*), 3.59 (1H, td, *J* 8.9, 2.3, Ins 5-*H*), 3.46 (1H, dd, *J* 9.5, 2.6, Ins 1-*H*), 3.45 (1H, td, *J* 8.8, 2.7, Ins 3-*H*), 2.44 (1H, d, *J* 2.4, Ins 5-O*H*), 1.68 (1H, d, *J* 8.4, Ins 3-O*H*), 1.21 (9H, s, SiC*Me*$_3$) ppm; δ_C (100 MHz, $CDCl_3$) 138.81, 138.61, 138.13 (3 × Ar *C*), 135.85 (2C), 135.82 (2C) (4 × Ar *C*H), 133.85 (2C) (2 × Ar *C*), 129.66, 129.61, 128.40 (2C), 128.32 (2C), 128.21 (2C), 127.86 (2C), 127.71 (2C), 127.69, 127.61 (2C), 127.59 (2C), 125.53, 127.31, 127.12 (2C) (21 × Ar *C*H), 81.12, 80.66, 77.59, 76.47, 75.53 (5 × Ins *C*H), 75.51, 74.39 (2 × O*C*H_2Ph), 72.82 (Ins *C*H), 72.73 (O*C*H_2Ph), 27.09 (SiC*Me*$_3$), 19.71 (Si*C*Me_3) ppm; HRMS (ESI+) *m*/*z* (%) found $[M+Na]^+$ 711.3106 (100), $C_{43}H_{48}O_6SiNa$ requires 711.3118.

2,3,6-*O*-Tribenzyl-4-*C*-methyl-*myo*-inositol (11). 2,6-*O*-Dibenzyl-4-*C*-methyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (**10**, 100 mg, 0.21 mmol) was reduced with DIBAL-H using the general method described above. The residue was fractionated by chromatography on flash silica. Elution with hexane-EtOAc (9:1 → 4:6 v/v) afforded **11** (55 mg, 55%) as a clear oil which crystallised on standing; R_f (hexane-EtOAc, 7:3 v/v) 0.09; mp 101–102 °C; δ_H (500 MHz, CD_3OD) 7.44–7.21 (15H, m, 15 × Ar *H*), 4.84–4.81 (3H, m, 6-OC*H*$_2$Ph, 2-OCH*H*Ph), 4.73 (1H, d, *J* 11.7, 3-OCH*H*Ph), 4.72 (1H, d, *J* 11.4, 2-OCH*H*Ph), 4.66 (1H, d, *J* 11.7, 3-OCH*H*Ph), 3.99 (1H, t, *J* 2.8, Ins 2-*H*), 3.55–3.50 (2H, m, Ins 1-*H* + Ins 6-*H*), 3.39 (1H, d, *J* 8.9, Ins 5-*H*), 3.37 (1H, d, *J* 2.8, Ins 3-*H*), 1.41 (3H, s, Ins 4-C*H*$_3$) ppm; δ_C (125 MHz, CD_3OD) 140.50, 140.40, 140.22 (3 × Ar *C*), 129.29 (2C), 129.45 (2C), 128.14 (2C), 129.10 (2C), 128.93 (2C), 128.83 (2C), 128.53, 128.46, 128.32 (15 × Ar *C*H), 84.04, 82.55, 79.23, 78.89 (4 × Ins *C*H), 78.24 (Ins *C*), 76.09, 75.88, 74.35 (3 × O*C*H_2Ph), 73.31 (Ins *C*H), 17.70 (Ins 4-*C*H_3) ppm; HRMS (CI+) *m*/*z* (%) found $[M+NH_4]^+$ 482.2545 (100), $C_{28}H_{36}NO_6$ requires 482.2542.

1,4,5-*O*-Tris(dibenzyloxyphosphoryl)-2,3,6-*O*-tribenzyl-4-*C*-methyl-*myo*-inositol

2,3,6-*O*-Tribenzyl-4-*C*-methyl-*myo*-inositol (**11**, 64 mg, 0.14 mmol) and 1-*H*-tetrazole (116 mg, 1.66 mmol) were evaporated from MeCN (3 × 2 mL), taken up in MeCN (5 mL) and *N*,*N*-diisopropyldibenzyl phosphoramidite (272 μL, 0.83 mmol) added. After 2 h the solution was cooled to –40 °C and *m*CPBA (222 mg, 0.97 mmol) added. Stirring was continued at 0 °C for 2 h. The solution was diluted with CH_2Cl_2, washed with 10% aq. $Na_2S_2O_3$, sat. $NaHCO_3$, H_2O and brine. The organic layer was dried ($MgSO_4$) and evaporated to dryness under reduced pressure. The residue was fractionated by chromatography on flash silica. Elution with hexane-EtOAc (3:1 → 0:1 v/v) afforded the *title compound* (97 mg, 57%) as a pale yellow oil; R_f (hexane-EtOAc, 7:3 v/v) 0.15; δ_H (400 MHz, $CDCl_3$) 7.42–7.02 (45H, m, 45 × Ar *H*), 5.12–4.68 [18H, m, (17 × Ph-CH*H*O) + Ins 5-*H*], 4.58–4.50 (3 H, m, OCH*H*Ph + Ins 1-*H* + Ins 2-*H*), 4.16 (1H, d, *J* 2.3, Ins 3-*H*), 4.00 (1H, t, *J* 9.6, Ins 6-*H*), 1.02 (3H, s, Ins 4-C*H*$_3$) ppm; δ_C (100 MHz, $CDCl_3$) 138.38, 138.21, 137.97 (3 × Ar *C*), 136.26 (d, *J* 7.8), 136.10 (d, *J* 7.8), 136.03 (d, *J* 6.0), 135.81 (d, *J* 7.1), 135.60 (d, *J* 6.5), 135.57 (d, *J* 6.6) (6 × Ar *C*CH$_2$OP), 128.56 (5C), 128.35 (5C), 128.29 (4C), 128.23 (5C), 128.13 (4C), 127.99 (3C), 127.92 (2C), 127.83 (2C), 127.76 (2C), 127.61 (2C), 127.58 (2C), 127.49, 127.44 (2C), 127.34, 127.27 (2C), 127.16 (3C) (45 × Ar *C*H), 89.35 (dd, Ins 4-*C*CH$_3$, *J* 8.2, 2.6), 82.73 (bs, Ins 5-*C*H), 78.40 (Ins 3-*C*H), 77.54 (d, *J* 5.6, Ins 1-*C*H), 77.09 (d, *J* 6.1, Ins 6-*C*H), 75.29 (2-O*C*H_2Ph), 74.59 (6-O*C*H_2Ph), 74.15 (Ins 2-*C*H), 70.84 (3-O*C*H_2Ph), 69.63 (d, *J* 5.2), 69.39 (d, *J* 5.2), 69.22 (2C, d, *J* 5.2), 69.03 (2C, d, *J* 5.1) (6 × PO*C*H_2Ph), 17.65 (d, *J* 2.1, Ins 4-*C*H_3) ppm; δ_P (162 MHz, $CDCl_3$) –1.88, –2.06, –7.23 ppm; HRMS (ES+) *m*/*z* (%) found $[M+H]^+$ 1245.4048 (100), $C_{70}H_{72}O_{15}P_3$ requires 1245.4084.

4-*C*-Methyl-*myo*-inositol 1,4,5-*O*-triphosphate (12a)

1,4,5-*O*-Tris(dibenzyloxyphosphoryl)-2,3,6-*O*-tribenzyl-4-*C*-methyl-*myo*-inositol (64 mg, 0.05 mmol) was taken up in ^{t}BuOH-H_2O (6:1 v/v) to which was added $NaHCO_3$ (46 mg, 0.41 mmol) and Pd-black (145 mg, 1.03 mmol). The solution was stirred under an atmosphere of H_2 for 36 h. The catalyst was filtered off, washed with H_2O (4 × 10 mL) and concentrated under reduced pressure, before being taken up in H_2O, washed with CH_2Cl_2 (× 2) and freeze dried. The powdery solid was re-dissolved in the minimum volume of H_2O and passed through DOWEX H-100 resin. Acidic fractions of eluent were combined, neutralised with aq. NH_3 and freeze dried to yield **12a** (30 mg, 100%) as a powdery salt; δ_H (400 MHz, D_2O) 4.20 (1H, t, *J* 3.1, Ins 2-*H*), 4.05 (1H, dd, *J* 9.5, 8.9, Ins 5-*H*), 3.98 (1H, ddd, *J* 9.6, 9.1, 3.0, Ins 1-*H*), 3.89 (1H, d, *J* 3.2, Ins 3-*H*), 3.75 (1H, dd, *J* 9.9, 9.8, Ins 6-*H*), 1.48 (3H, s, Ins 4-C*H*$_3$) ppm; δ_C (125 MHz, D_2O) 87.76 (d, Ins 4-*C*CH$_3$, *J* 7.0), 83.82 (t, *J* 6.8), 77.65 (d, *J* 5.3), 75.59 (s), 72.59 (s), 72.33 (s) (5 × Ins *C*H), 17.81 (s, Ins 4-*C*H$_3$) ppm; δ_P (162 MHz, D_2O) 2.55, 2.31, 0.30 ppm; HRMS (ES^-) *m*/*z* (%) found $[M-H]^-$ 432.9716 (100), $C_7H_{16}O_{15}P_3$ requires 432.9702.

1,3,4,5-*O*-Tetrakis(dibenzyloxyphosphoryl)-2,6-*O*-dibenzyl-4-*C*-methyl-*myo*-inositol

2,6-*O*-Dibenzyl-4-*C*-methyl-*myo*-inositol (**13**, 75 mg, 0.20 mmol) and 1-*H*-tetrazole (196 mg, 2.80 mmol) were evaporated from MeCN (3 × 2 mL), taken up in MeCN (5 mL) and *N*,*N*-diisopropyldibenzyl phosphoramidite (461 μL, 1.40 mmol) added. After 2 h the solution was cooled to –40 °C and *m*CPBA (644 mg, 2.80 mmol, 75%) added. Stirring was continued at 0 °C. After 2 h the solution was diluted with CH_2Cl_2, washed with 10% aq. $Na_2S_2O_3$, sat. $NaHCO_3$, H_2O and brine. The organic layer was dried ($MgSO_4$) and evaporated to dryness under reduced pressure. The residue was fractionated by chromatography on flash silica. Elution with hexane-EtOAc (3:1 → 0:1: v/v) afforded the *title compound* (178 mg, 63%) as a clear oil; R_f (EtOAc-CH_2Cl_2, 1:4 v/v) 0.52; δ_H (400 MHz, $CDCl_3$) 7.41–7.01 (50H, m, 50 × Ar *H*),

5.12–4.67 [25H, m, (20 × Ph-CH*H*O) + (5 × Ins *H*)], 1.31 (3H, s, Ins 4-C*H*$_3$) ppm; δ_P (162 MHz, D_2O) –1.46, –1.65, –1.86, –6.30 ppm; MS (MALDI-TOF) *m*/*z* (%) found $[M+Na]^+$ 1438 (40).

4-*C*-Methyl-*myo*-inositol 1,3,4,5-*O*-tetraphosphate (12b)

1,3,4,5-*O*-Tetrakis(dibenzyloxyphosphoryl)-2,6-*O*-dibenzyl-4-*C*-methyl-*myo*-inositol (350 mg, 0.25 mmol) was taken up in ^{t}BuOH-H_2O (54 mL, 6:1 v/v) to which was added $NaHCO_3$ (166 mg, 1.98 mmol) and Pd-black (527 mg, 4.95 mmol). The solution was stirred under an atmosphere of H_2 for 36 h. The catalyst was filtered off, washed with H_2O (4 × 10 mL) and the filtrate concentrated under reduced pressure. The remaining solution was taken up in H_2O, washed with CH_2Cl_2 (× 2) and freeze dried. The powdery solid was re-dissolved in the minimum volume of H_2O and passed through DOWEX H-100 resin. Acidic fractions of eluent were combined, neutralised with aq. NH_3 and freeze dried to yield **12b** (69 mg, 100%) as a pale brown powdery salt; δ_H (500 MHz, D_2O) 4.23 (1H, t, *J* 3.4, Ins 2-*H*), 4.14 (1H, dd, *J* 10.1, 3.4, Ins 3-*H*), 4.02 (1H, t, *J* 9.5, Ins 5-*H*), 3.87 (1H, ddd *J* 10.1, 8.5, 3.3, Ins 1-*H*), 3.6 (1H, t, *J* 9.9, Ins 6-*H*), 1.44 (3H, s, Ins 4-C*H*$_3$) ppm; δ_C (125 MHz, D_2O) 84.05 (dt, *J* 6.8, 4.9, Ins 4-*C*CH$_3$), 81.28 (bm), 76.69 (d, *J* 5.7), 73.92 (d, *J* 5.2), 70.48 (s), 70.21 (bm) (5 × Ins *C*H), 15.21 (s, Ins 4-*C*H$_3$) ppm; δ_P (162 MHz, D_2O) 7.74, 1.38, 0.83, –2.35 ppm; HRMS (ESI^-) *m*/*z* (%) found $[M-H]^-$ 512.9350 (77), $C_7H_{17}O_{18}P_4$ requires 512.9365.

2,6-*O*-Dibenzyl-4-*C*-methyl-*myo*-inositol (14)

2,6-*O*-Dibenzyl-4-*C*-methyl-*myo*-inositol 1,3,5-*O*-orthobenzoate (**10**, 200 mg, 0.43 mmol) was refluxed in conc. HCl-methanol (6 mL, 1:2 v/v). After 3 h the solution was cooled, diluted with H_2O and extracted with EtOAc. The organic layer was washed with sat. $NaHCO_3$, H_2O, and then brine, dried ($MgSO_4$) and all solvents evaporated under reduced pressure. ^{1}H NMR showed that more than one isomeric product was present, including **13a**. If desired **13a** may be isolated by chromatography on flash silica, eluting with EtOAc-hexane (1:4 → 3:2 v/v); δ_H (400 MHz, CD_3OD) 7.93–7.06 (15H, m, 15 × Ar C*H*), 5.11 (1H, dd, *J* 10.1, 3.3, Ins 1-*H*), 4.84 (1H, d, *J* 11.1, PhCH*H*O), 4.81 (1H, d, *J* 11.7, PhCH*H*O), 4.68 (1H, d, *J* 11.1, PhCH*H*O), 4.59 (1H, d, *J* 11.7, PhCH*H*O), 4.10 (1H, t, *J* 3.1, Ins 2-*H*), 3.90 (1H, t, *J* 10.0, Ins 6-*H*), 3.67 (1H, d, *J* 3.0, Ins 3-*H*), 3.52 (1H, d, *J* 9.8, Ins 5-*H*), 1.41 (3H, s, Ins 4-C*H*$_3$) ppm; δ_C (100 MHz, CD_3OD) 167.26 (Ph*C*O$_2$), 140.13, 139.80 (2 × Ar *C*), 134.41 (2 × Ar *C*H), 131.13 (Ar *C*), 130.80 (3C), 129.57 (2C), 129.17, 129.08 (3C), 128.55 (2C), 128.47, 128.36 (13 × Ar *C*H), 80.21, 79.86, 79.12 (3 × Ins *C*H), 77.70 (Ins 4-*C*CH$_3$), 76.40, 76.17 (2 × Ph*C*H$_2$O), 75.56, 75.30 (2 × Ins *C*H), 16.97 (Ins 4-C*C*H$_3$). The crude mixture of isomeric benzoates (180 mg, 0.38 mmol) was evaporated from MeCN (3 × 1 mL) and taken up in MeOH (2 mL). NaOMe (25% solution in MeOH, 52 µL, 0.5 eq) was added and the reaction refluxed for 3 h. After careful neutralisation with 4 M HCl the solvent was evaporated. The residue was taken up in methanol, the solids removed by filtration, and the mother-liquor evaporated to dryness. The crude material was fractionated by chromatography on flash silica. Elution with CH_2Cl_2-hexane (0:1 → 1:0 v/v) then EtOH-CH_2Cl_2 (0:1 → 3:97 v/v) afforded **14** (139 mg, 87%) as a clear oil; δ_H (400 MHz, CD_3OD) 7.46–7.25 (10H, m, 10 × Ar *H*), 4.89 (1H, d, *J* 11.3, OC*H*HPh), 4.85–4.80 (3H, m, 3 × OC*H*HPh), 3.90 (1H, t, *J* 3.1, Ins 2-*H*), 3.67 (1H, dd *J* 9.6, 3.2, Ins 1-*H*), 3.60–3.55 (2H, m, Ins 6-*H* + Ins 3-*H*), 3.43 (1H, d, *J* 9.4, Ins 5-*H*), 1.36 (3H, s, Ins 4-C*H*$_3$) ppm; δ_C (100 MHz, $CDCl_3$) 139.15, 139.04 (2 × Ar *C*), 127.89 (2C), 127.79 (4C), 127.53 (2C), 127.13, 126.97 (10 × Ar *C*H), 81.26, 80.72, 77.39 (3 × Ins *C*H), 76.35 (Ins *C*), 75.10 (O*C*HPh), 74.58 (Ins *C*H), 74.39 (O*C*HPh), 72.05 (Ins *C*H), 15.88 (4-*C*H$_3$) ppm; HRMS (ESI^+) *m*/*z* (%) found $[M+Na]^+$ 397.1628 (100), $C_{21}H_{26}O_6Na$ requires 397.1627.

Acknowledgements

Funding from the UK Medical Research Council (PRJG, grant no. G 120/709) and an EPSRC doctoral training award to the Chemical Biology Centre, Imperial College (SC) are gratefully acknowledged. GB is a postdoctoral fellow of the Research Foundation, Flanders (F.W.O.) and would like to thank Tomas Luyten for technical assistance.

References

1 (*a*) R. F. Irvine and M. J. Schell, *Nat. Rev. Mol. Cell. Biol.*, 2001, **2**, 327–38; (*b*) M. Berridge and R. Irvine, *Nature*, 1989, **341**, 197–205.
2 (*a*) B. V. L. Potter and D. Lampe, *Angew. Chem., Int. Ed.*, 1995, **34**, 1933–1972; (*b*) K. M. Sureshan, M. S. Shashidhar, T. Praveen and T. Das, *Chem. Rev.*, 2003, **103**, 4477–4503.
3 (*a*) K. M. Sureshan and Y. Watanabe, *Tetrahedron: Asymmetry*, 2004, **15**, 1193–1198; (*b*) M. S. Shashidhar, *ARKIVOC*, 2002, **7**, 63–75.
4 H. W. Lee and Y. Kishi, *J. Org. Chem.*, 1985, **50**, 4402–4404.
5 T. Praveen and M. S. Shashidhar, *Carbohydr. Res.*, 2001, **330**, 409–411.
6 A. M. Riley, Y. H. Godage, M. F. Mahon and B. V. L. Potter, *Tetrahedron: Asymmetry*, 2006, **17**, 171–174.
7 M. P. Sarmar and M. S. Shashidhar, *Carbohydr. Res.*, 2003, **338**, 999–1001.
8 I. H. Gilbert, A. B. Holmes, M. J. Pestchanker and R. C. Young, *Carbohydr. Res.*, 1992, **234**, 117–130.
9 C. Maurali, M. S. Shashidhar and C. S. Gopinath, *Tetrahedron*, 2007, **63**, 4149–4155.
10 D. C. Billington and R. Baker, *J. Chem. Soc., Chem. Commun.*, 1987, **13**, 1011–1013.
11 J. Gigg and R. Gigg, *J. Chem. Soc. C*, 1966, **1**, 82–86.
12 (*a*) K. L. Yu and B. Fraser-Reid, *Tetrahedron Lett.*, 1988, **29**, 979–982; (*b*) P. Westerduin, H. A. M. Willems and C. A. A. van Boeckel, *Carbohydr. Res.*, 1992, **234**, 131–140.
13 S. W. Garrett, C. Liu, A. M. Riley and B. V. L. Potter, *J. Chem. Soc., Perkin Trans. 1*, 1998, **8**, 1367–1368.
14 S. Devaraj, M. S. Shashidhar and S. S. Dixit, *Tetrahedron*, 2005, **61**, 529–536.
15 C. H. Marzabadi, E. J. Anderson, J. Gonzalez-Outeirino, P. R. J. Gaffney, C. G. H. White, D. A. Tocher and L. J. Todaro, *J. Am. Chem. Soc.*, 2003, **125**, 15163–73.
16 M. P. Sarmah, M. S. Shashidhar, K. M. Sureshan, R. G. Gonnade and M. M. Bhadbhade, *Tetrahedron*, 2005, **61**, 4437–4446.
17 K. M. Sureshan, A. M. Riley and B. V. L. Potter, *Tetrahedron Lett.*, 2007, **48**, 1923–1926.
18 O. Bouab, G. Lamaty, C. Moreau, O. Pomares, P. Deslongchamps and L. Ruest, *Can. J. Chem.*, 1980, **58**, 567–573.
19 A. Miyawaki, T. Furuichi, N. Maeda and K. Mikoshiba, *Neuron*, 1990, **5**, 11–18.
20 G. Bultynck, K. Szlufcik, N. N. Kasri, Z. Assefa, G. Callewaert, L. Missiaen, J. B. Parys and H. De Smedt, *Biochem. J.*, 2004, **381**, 87–96.