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Synthesis of anti-tumour phosphatidylinositol analogues from glucose by the use of ring-closing olefin metathesis†

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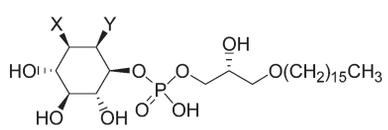
A divergent strategy is described for synthesis of the novel phosphatidylinositols **1**–**3**. The synthetic approach commences from benzyl-protected methyl 6-iodo-6-deoxy-α-D-glucopyranoside, which undergoes zinc-mediated reductive fragmentation followed by vinyl Grignard addition and ring-closing metathesis to afford the key conduritol B intermediate **7**. This can trifurcate to form three different benzyl-protected *myo*-inositol headgroups **4**–**6**, which after phosphorylation and attachment of the glycerolipid part give phosphatidylinositols **1**–**3**. Preliminary biological testing against human colon adenocarcinoma cells reveals that analogues **1**–**3** are significant anti-tumour agents.

Introduction

Phosphatidylinositols (PtdIns) are involved in a number of important cellular processes including cell proliferation, intracellular vesicle trafficking and cell metabolism.[1] They are known to undergo phosphorylation at multiple sites to generate diverse phosphoinositides (PIs) that regulate a series of signal transduction pathways.[2] PtdIns can undergo sequential and reversible phosphorylations on the D-3, D-4, and D-5 positions by specific kinases. PtdIns(4,5)P_2 is hydrolysed by a phosphatidylinositol-specific phospholipase C (PI-PLC) which generates two important second messengers, Ins(1,4,5)P_3 and diacylglycerol.[3,4] This initiates an important pathway for cell proliferation where Ins(1,4,5)P_3 interacts with membrane receptors to release Ca^{2+}, which is a key event in cellular signal transduction.[5] PtdIns are also phosphorylated by a family of PI 3-kinases that displays different activity towards various PtdIns substrates. PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3 are rapidly produced by PI 3-kinase (PI3K) in response to agonist-mediated cell stimulation.[1] These metabolites are poor substrates for PI-PLC and appear to be important modulators of cell growth through protein interaction and specific binding to enzymes such as Akt (protein kinase B).[6] Akt becomes fully activated upon phosphorylation by PtdIns dependent kinases, and it regulates cell survival and proliferation by phosphorylating a number of downstream targets which are involved in apoptosis.[7]

Cancer cells escape normal growth control mechanisms by inactivating cell growth regulating enzymes and tumour suppressor genes, or by activating/mutating cellular protooncogenes. High levels of active Akt have been found in many types of human tumours[8] and a correlation has been found between high levels of Akt and defects in regulatory phosphatase PTEN.[9] To obtain a better understanding of Akt in cancer biology, it has been of interest to develop inhibitors directed against Akt, which may further lead to the development of novel anti-cancer drugs. It has been shown that phosphatidyl 3-deoxy-inositols are effective Akt inhibitors and that these compounds are potential therapeutic candidates for use in cancer therapy.[10]

Anti-tumour ether lipids (AELs) are a promising class of anti-tumour agents that induce apoptosis by several different mechanisms,[11] *e.g.* by effecting the PI3K regulated Akt pathway.[12] However, AELs have been limited in use due to their hemolytic properties[11] and drug delivery formulations have been proposed to protect the red blood cells from hemolysis.[13] Kozikowski and co-workers have obtained a new class of potent anti-tumour drugs through the synthesis of ether lipids bearing inositol derivatives as headgroups.[6] These ether lipids have been shown to specifically inhibit Akt phosphorylation.[10] We have been interested in AELs and masked AELs (proAELs) with inositol headgroups because we anticipate that these new lipids can be formulated in a drug delivery system with phospholipase A_2 as a site-specific trigger.[14] Phospholipase A_2 hydrolyses the fatty acid ester in the *sn*-2 position of proAELs and releases the active AELs specifically in the tumour tissue.[14] In this paper, we have directed our attention toward PtdIns **1**, **2**, and **3** as active AEL targets (Fig. 1). Compounds **1** and **2** are deoxygenated at the D-2 and/or D-3 positions in the *myo*-inositol part while compound **3** is the parent PtdIns for comparison of the biological data.



1: X = Y = H
2: X = H, Y = OH
3: X = Y = OH

Fig. 1 Structures of target PtdIns **1**–**3**.

The primary synthetic challenge lies in the synthesis of the three different inositol headgroups. Previously, enantiopure *myo*-inositol derivatives have been prepared by resolution of *myo*-inositol,[15] catalytic enantioselective syntheses[16] and by carbocyclisation of carbohydrates.[17] In the latter case, the key cyclisation reaction is either a Ferrier reaction, a samarium mediated pinacol coupling or an olefin metathesis reaction. In the past decade, alkene metathesis reactions catalysed by ruthenium carbene complexes have had a major impact on organic chemistry.[18] We have recently described several strategies for converting carbohydrates into functionalised carbocycles by the use of ring-closing metathesis.[19,20] These procedures have also been applied in the synthesis of carbocyclic natural products.[21]

Herein, we report the chemical synthesis of PtdIns **1**, **2**, and **3**, where the inositol headgroups have been prepared from a carbohydrate by olefin metathesis. Preliminary biological testing has been performed against human colon cancer cells.

Results and discussion

Retrosynthesis

The glycerolipid portion of **1**–**3** will be prepared from (*R*)-glycidyl tosylate and introduced by a phosphorylation reaction with

† Electronic supplementary information (ESI) available: ^1H and ^{13}C NMR data for compounds **4**–**6** and **7R** as well as spectra of compounds **1**–**3**. See http://www.rsc.org/suppdata/ob/b4/b411021h

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inositol derivatives **4**–**6** (Fig. 2). We envisage assembling these three cyclitols by a divergent strategy from D-glucose. It should be noticed that the stereochemistry at positions 4–6 in the three inositol headgroups is identical to the stereochemistry at positions 2–4 in D-glucose. Accordingly, each of the compounds **4**–**6** will be constructed from the same intermediate **7**. Cyclohexene **7** is a protected derivative of conduritol B and will be prepared by ring-closing olefin metathesis from diene **8**. The two double bonds in **8** will be introduced by a zinc-mediated fragmentation of iodoglucoside **10** followed by addition of a vinyl group to the formed aldehyde **9**.[19c]

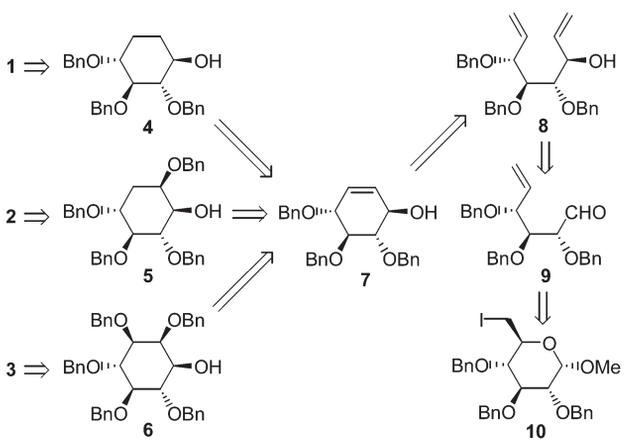
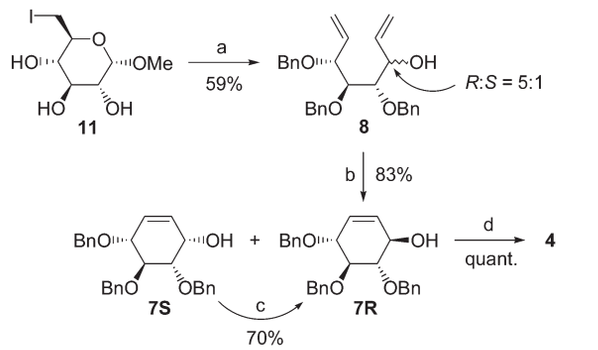


Fig. 2 Retrosynthesis for PtdIns **1**–**3**.

Synthesis of cyclohexane 4

The required starting material, methyl 6-iodo-6-deoxy-α-D-glucopyranoside (**11**), is readily available by regioselective iodination of the parent methyl glucoside.[22] Benzylation of **11** was performed under acidic conditions with benzyl trichloroacetimidate to afford **10**[23] which after workup to remove excess benzylating agent was subjected to sonication with zinc metal (Scheme 1).[23] The obtained aldehyde **9** was separated from the zinc salts by extraction and then reacted directly with vinyl organometallic reagents. When commercially available vinyl magnesium bromide was added to a THF solution of **9** at −78 °C, the desired product **8** was obtained in 57% yield (from **11**) as a 1 : 1 diastereomeric mixture. Unfortunately, the addition of divinyl zinc to **9** at 0 °C did not provide any stereocontrol either although the yield increased to 65% (from **11**). This came as a surprise since divinyl zinc has previously given very good selectivity in addition to carbohydrate aldehydes including protected glucose derivatives.[19c,24] We then turned our attention to the work of Kornienko and d'Alarcao, who have described vinyl Grignard addition to the enantiomer of **9**.[25] The reaction is performed in a dichloromethane–THF mixture at −78 °C to afford a 3 : 1 ratio of the two diastereomers. In fact, when we added vinyl magnesium bromide to a solution of **9** in dichloromethane, diene **8** was obtained in 55% yield (from **11**) as a 5 : 2 mixture of the (*R*)- and the (*S*)-isomer. Clearly, the non-polar co-solvent favours the formation of the desired (*R*)-diastereomer. However, we were uncomfortable with the use of dichloromethane in a Grignard reaction and found that the same yield and selectivity could be obtained in toluene.

It appears that the magnesium salts formed during the reaction have a significant impact on the diastereoselectivity. Kornienko and d'Alarcao have shown that the selectivity changes to 1 : 8 in favour of the opposite diastereomer when the Grignard addition is performed in the presence of 3 equiv. of magnesium dibromide etherate.[25] The reason for the improved selectivity for the (*R*)-isomer in unpolar solvents could be that the generated magnesium salts precipitate under these conditions. Therefore, we speculated that the counterion may play a significant role in this reaction. As a matter of fact, when we performed the Grignard reaction in toluene with commercially available

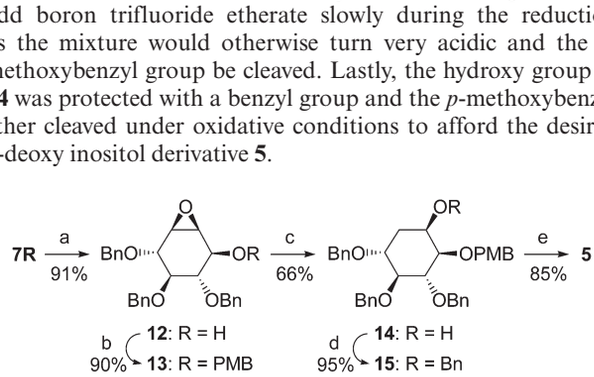


Scheme 1 *Reagents and conditions*: (a) $Cl_3CC(NH)OBn$, TfOH, dioxane, rt, then Zn, THF, H_2O, ultrasound, 40 °C, then CH_2=CHMgCl, THF, toluene, −78 °C; (b) 5% $(PCy_3)_2Cl_2Ru$=CHPh, CH_2Cl_2, rt; (c) Dess–Martin periodinane, CH_2Cl_2, rt, then $NaBH_4$, $CeCl_3 \cdot 7H_2O$, MeOH, 0 °C; (d) H_2, Pd/C, EtOAc.

vinyl magnesium *chloride*, the ratio between the (*R*)- and the (*S*)-isomer changed to 5 : 1. The two diastereomers of **8** were isolated in 59% yield (from **11**), but could not be separated by silica gel chromatography. Consequently, the ensuing metathesis reaction was performed on the diastereomeric mixture to furnish the corresponding cyclohexenes **7R** and **7S**, which could now be separated by flash chromatography. Furthermore, the undesired isomer **7S** could be converted into **7R** by a one-pot oxidation–reduction[26] sequence in 70% yield. The dideoxy inositiol derivative **4** was then available from **7R** by saturation of the double bond in quantitative yield.

Synthesis of cyclohexane 5

Constructing 3-deoxy derivative **5** from cyclohexene **7R** called for the introduction of a benzyloxy group in a regio- and diastereoselective manner. A hydroxyl directed epoxidation was chosen to ensure the necessary diastereoselectivity. The regioselectivity will then be governed by a subsequent ring-opening of this epoxide in a *trans*-diaxial fashion. Indeed, epoxidation of **7R** with *m*-CPBA furnished epoxyalcohol **12**,[26] which was subsequently converted into *p*-methoxybenzyl ether **13** (Scheme 2). Ring-opening of the epoxide was achieved in the presence of sodium borohydride and boron trifluoride etherate[27] to give a 3 : 1 mixture of the two regioisomeric alcohols. These could be separated by flash chromatography and the desired alcohol **14** was isolated in 66% yield. It was important to add boron trifluoride etherate slowly during the reduction as the mixture would otherwise turn very acidic and the *p*-methoxybenzyl group be cleaved. Lastly, the hydroxy group in **14** was protected with a benzyl group and the *p*-methoxybenzyl ether cleaved under oxidative conditions to afford the desired 3-deoxy inositol derivative **5**.

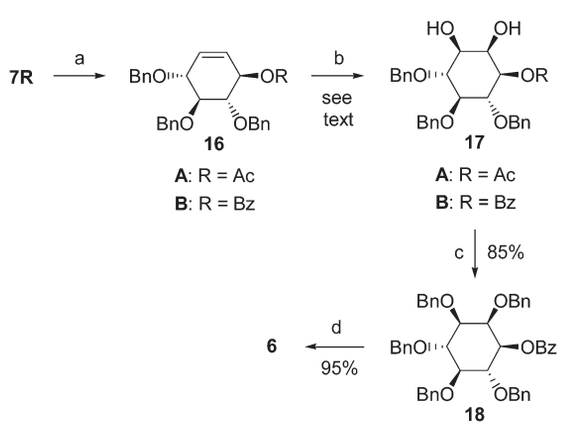


Scheme 2 *Reagents and conditions*: (a) *m*-CPBA, CH_2Cl_2, rt; (b) PMBCl, NaH, DMF, rt; (c) $NaBH_4$, $BF_3 \cdot OEt_2$, DME, rt; (d) BnBr, NaH, DMF, rt; (e) DDQ, CH_2Cl_2, H_2O, rt.

Synthesis of cyclohexane 6

The synthesis of pentabenzylated *myo*-inositol **6** from cyclohexene **7R** required two benzyloxy groups to be installed in a stereocontrolled fashion. This could be achieved through an osmium tetroxide-catalysed *cis*-dihydroxylation. The allylic hydroxy group in **7R** would have to be protected as the dihydroxylation would otherwise generate a *meso* compound. It is known from previous work on conduritol B derivatives

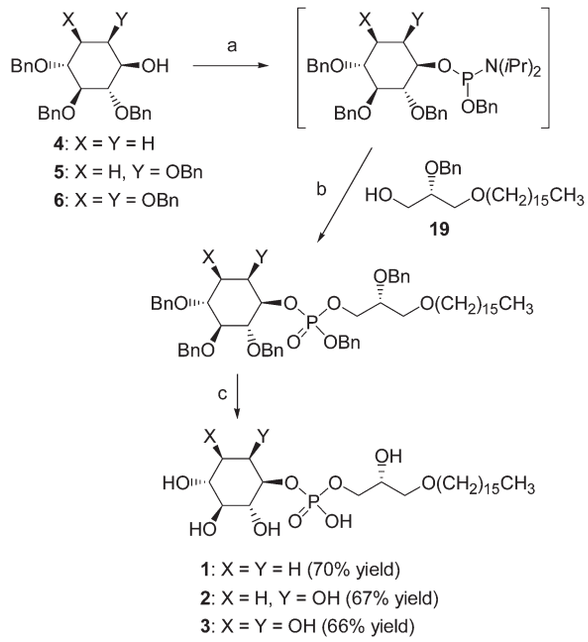
that ether protecting groups in both allylic positions give rise to poor diastereoselectivity in the dihydroxylation.[17b] However, Arjona and co-workers have demonstrated that if an ester group is present in one of the allylic positions and an ether group in the other, the osmium-catalysed dihydroxylation will mainly occur *syn* to the ester group.[28] Therefore, it was decided to convert **7R** into the allylic acetate **16A** (Scheme 3). Initially, the dihydroxylation of **16A** was performed with 5% of osmium tetroxide and *N*-methylmorpholine *N*-oxide as the stoichiometric oxidant. However, the reaction was very slow under these conditions and would not go to completion. Quinuclidine was then added as a ligand for osmium in order to accelerate the dihydroxylation. This gave complete conversion in about 24 h and diol **17A** was isolated in 91% yield as a 5 : 1 mixture of diastereomers. The major diastereomer was the desired isomer, which was confirmed after removing the acetate to afford the corresponding *meso* compound. The following benzylation, however, turned out to be problematic due to competing migration and cleavage of the acetate. Mixtures of several products were obtained when attempting to benzylate **17A** under basic conditions with benzyl bromide or under acidic conditions with benzyl trichloroacetimidate. It was therefore decided to employ a more stable ester group. Thus, treatment of **7R** with benzoyl chloride gave benzoate **16B**. Small amounts of benzoic anhydride could not be removed from **16B**, which was therefore taken on directly to the following dihydroxylation reaction. Gratifyingly, this reaction now produced the desired diol **17B** as a single diastereomer in high yield (87% from **7R**). None of the other diastereomer was observed in the dihydroxylation reaction. Benzylation of **17B** with benzyl bromide, sodium hydride, and tetrabutylammonium iodide in dimethylformamide gave fully protected derivative **18** in 44% yield. Again, the ester functionality was not fully compatible with the basic reaction conditions. Fortunately, this problem was solved by changing to acidic benzylation conditions, which now gave **18** in 85% yield. Removal of the benzoate by transesterification then afforded the protected *myo*-inositol **6**.



Scheme 3 *Reagents and conditions*: (a) BzCl, DMAP, Et_3N, CH_2Cl_2, rt; (b) OsO_4, NMO, quinuclidine, CH_2Cl_2, rt; (c) $Cl_3CC(NH)OBn$, TfOH, dioxane, rt; (d) MeONa, MeOH, rt.

Phosphorylation

Having completed the preparation of benzyl-protected *myo*-inositol derivatives **4**–**6** attention was then shifted toward the phosphorylation reaction and attachment of the lipid chain. The required dialkyl glycerol component **19** can easily be prepared in optically pure form by literature procedures.[29] The phosphorylations were performed by using the phosphoramidite method.[30] The protected inositols **4**–**6** were first reacted with commercially available benzyl-*N*,*N*-diisopropylchlorophosphoramidite[31] to afford the corresponding inositol amidites, which were not purified, but coupled directly with glycerol derivative **19** in the presence of 5-phenyl-1*H*-tetrazole (Scheme 4).[32] The obtained phosphites were then oxidised to the phosphates by addition of *tert*-butylhydroperoxide. The



Scheme 4 *Reagents and conditions*: (a) $BnO(Cl)PN(iPr)_2$, 2,2,6,6-tetramethylpiperidine, CH_2Cl_2, rt; (b) **19**, 5-phenyl-1*H*-tetrazole, CH_2Cl_2, rt, then *t*-BuOOH, 0 °C; (c) H_2, Pd/C, *t*-BuOH.

protected phosphatidylinositols thus formed were purified by chromatography, but not characterised in detail due to the diastereomeric mixture at phosphorous. Instead, the benzyl groups were removed by hydrogenolysis to give the desired analogues **1**–**3** in 66–70% overall yields from inositols **4**–**6**. The amphiphilic molecules **1**–**3** were isolated as amorphous solids and fully characterised by ^1H, ^{13}C, and ^{31}P NMR spectroscopy as well as mass spectrometry.

Biological testing and conclusion

A preliminary cytotoxic assay (MTT) was performed in order to evaluate the three phosphatidylinositols as anti-tumour agents. Human colon adenocarcinoma cells (HT-29) were incubated with PtdIns **1**–**3** for 72 hours. PtdIns **1** showed an IC_{50} of approximately 18 μM, whereas PtdIns **2** and **3** gave IC_{50} values in the range of 50–60 μM.

In conclusion, we have described the synthesis of three novel phosphatidylinositols, which have promising anti-tumour activity. The different inositol headgroups have been prepared from D-glucose by a divergent strategy. The inositol syntheses highlight the utility of our previously developed method for converting carbohydrates into carbocycles, where a zinc-mediated fragmentation, a vinyl Grignard addition, and a ring-closing metathesis reaction serve as the key steps. The PtdIns **1**–**3** are currently under further biological testing and prodrug analogues are being developed for *in vivo* use as part of a liposomal drug delivery system.[14]

Experimental

General

Dioxane, THF, and toluene were distilled from sodium benzophenone while CH_2Cl_2 was distilled from CaH_2. Thin-layer chromatography was performed on aluminium plates precoated with silica gel. Compounds were visualized by heating after dipping in a solution of $Ce(SO_4)_2$ (2.5 g) and $(NH_4)_6Mo_7O_{24}$ (6.25 g) in 10% aqueous H_2SO_4 (250 mL). Flash chromatography was performed with silica gel 60. NMR spectra were recorded on a Varian Unity Inova 500 or a Varian Mercury 300 spectrometer. Me_4Si (δ_H = 0.0 ppm) was used as the internal reference for ^1H NMR while $CDCl_3$ (δ_C = 77.1 ppm) served as the reference for ^{13}C NMR. ^{31}P NMR was carried out with an external reference of 85% aqueous H_3PO_4 (δ_P = 0.0 ppm). Optical rotations were

measured on a Perkin-Elmer 241 polarimeter. Microanalyses and high resolution mass spectra were obtained at the Department of Chemistry, University of Copenhagen.

(4*S*,5*S*,6*R*)-4,5,6-Tris(benzyloxy)octa-1,7-dien-3-ol (8)

Methyl 6-deoxy-6-iodo-α-D-glucopyranoside (**11**)[22] (5.0 g, 16.5 mmol) and benzyl trichloroacetimidate (13.8 mL, 75 mmol) were dissolved in dry dioxane (50 mL) and stirred under argon. Triflic acid was added dropwise until the mixture became strongly acidic (~60 drops) after which the solution turned dark red. The mixture was stirred for 45 min. TLC (EtOAc–hexane, 1:9) indicated that the reaction had gone to completion. Et_2O (150 mL) was added and the solution was washed with saturated aqueous $NaHCO_3$ (2 × 50 mL), water (2 × 50 mL), and brine (50 mL) after which it was dried with Na_2SO_4 and concentrated. The resulting oil was concentrated with silica and added to a short silica column (8 cm) and eluted with EtOAc–hexane, 1:6. This afforded glucopyranoside **10** as a white solid, which was used directly in the next step. To a solution of **10** in THF (70 mL) and H_2O (7 mL) was added activated zinc dust[19a,c] (10.8 g, 165 mmol) and the mixture was then sonicated for 5 h at 40 °C in a sonic bath. To the suspension were added Et_2O (70 mL) and water (30 mL), and the mixture was filtered through Celite. The layers were separated and the aqueous phase was extracted with Et_2O (2 × 50 mL). The combined organic phases were washed with water (2 × 50 mL) and brine (50 mL), and then dried with Na_2SO_4 and concentrated. The crude product was co-evaporated with toluene (3 × 50 mL) to give aldehyde **9** as a syrup, which was used in the next step without further purification. This material was dissolved in dry THF (5 mL) under argon. Toluene (1 L) was then added followed by slow addition of 1.6 M vinyl magnesium chloride (100 mL) over 30 min at −78 °C. The yellow solution was stirred at −78 °C for 1.5 h after which time TLC (EtOAc–hexane, 1:5) indicated that the reaction had gone to completion. The mixture was quenched by the addition of water (50 mL) followed by 1 M aqueous HCl (300 mL). The organic phase was separated and washed with water (2 × 300 mL) and brine (300 mL), and then dried with Na_2SO_4 and concentrated. The crude oil was purified by flash chromatography (EtOAc–hexane, 1:6) to give 4.32 g (59%) of **8** as a 5:1 mixture of diastereomers, which were separated in the next step. R_f 0.26 (EtOAc–hexane, 1:5); δ_C (75 MHz, $CDCl_3$, major isomer): 138.6–137.7 (3C), 135.3, 135.2, 129.0–127.7 (15C), 119.1, 115.6, 82.1, 81.9, 80.0, 75.3, 75.1, 72.0, 70.4; δ_C (75 MHz, $CDCl_3$, minor isomer): 138.6–137.7 (3C), 135.3, 135.2, 129.0–127.7 (15C), 119.3, 116.3, 81.7, 81.4, 80.1, 74.7, 72.6, 71.9, 70.7.

(4*S*,5*S*,6*S*)-4,5,6-Tris(benzyloxy)cyclohex-2-enol (7)

Diene **8** (3.30 g, 7.40 mmol) was dissolved in CH_2Cl_2 (100 mL) and a stream of argon was passed through the solution for 30 min. $(PCy_3)_2Cl_2Ru{=}CHPh$ (0.30 g, 0.37 mmol) was then added to the deoxygenated solution and the reaction was stirred under argon for 16 h. To the black solution was added 1.5 M aqueous tris(hydroxymethyl)phosphine (6.5 mL) and the mixture was stirred overnight giving a yellow solution. The phases were separated and the organic phase was washed with water (3 × 40 mL), dried with $MgSO_4$, and concentrated. Purification by column chromatography (Et_2O–hexane, 1:2) gave 2.11 g (69%) of **7R** as a white solid and 0.43 g (14%) of the undesired diastereomer **7S**.

For **7R**. R_f 0.26 (EtOAc–hexane, 1:3); mp 114–115 °C (Et_2O–hexane, 1:1) (lit.[33] mp 122 °C); $[\alpha]_D$ −119 (*c* 1.4, $CHCl_3$) (lit.[33] $[\alpha]^{23}_D$ −116.0 (*c* 1.87, $CHCl_3$)); Anal. calcd. for $C_{27}H_{28}O_4$: C, 77.86; H, 6.78. Found: C, 77.77; H, 6.76%.

(1*R*,4*R*,5*S*,6*S*)-4,5,6-Tris(benzyloxy)cyclohex-2-enol (7R)

Dess–Martin periodinane (3.18 g, 7.49 mmol) was added to a solution of **7S** (2.59 g, 6.24 mmol) in CH_2Cl_2 (40 mL). The suspension was stirred for 1.5 h under argon after which time TLC (EtOAc–hexane, 1:3) showed that the reaction had gone to completion. Et_2O (50 mL) was added and the mixture stirred for 15 min, filtered and concentrated. The crude product was dissolved in MeOH (30 mL) followed by careful addition of $CeCl_3{\cdot}7H_2O$ (2.56 g, 6.86 mmol) and $NaBH_4$ (472 mg, 12.5 mmol) at 0 °C. The reaction was stirred for 2 h at 0 °C and then allowed to warm to room temperature. Acetone (20 mL) was added and the mixture stirred for 15 min, filtered and concentrated. The residue was dissolved in Et_2O (120 mL) and washed with water (3 × 40 mL). The combined aqueous phases were extracted with EtOAc (50 mL). The combined organic phases were dried with Na_2SO_4 and concentrated to afford a white solid. Recrystallisation from Et_2O–hexane gave 1.81 g (70%) of **7R** as white crystals.

1D-4,5,6-Tri-*O*-benzyl-2,3-dideoxy-*myo*-inositol (4)

Cyclohexene **7R** (240 mg, 0.58 mmol) was dissolved in EtOAc (20 mL) and hydrogenated for 2.5 h at 1 atm. with 10% Pd/C (24 mg) as catalyst. The reaction was monitored by TLC (EtOAc–hexane, 1:3) and upon completion the mixture was filtered and concentrated to give 240 mg (quant.) of **4** as white crystals. R_f 0.20 (EtOAc–hexane, 1:3); mp 84–85 °C (hexane) (lit.[34] mp 87–88.5 °C); $[\alpha]_D$ −26.5 (*c* 1.4, $CHCl_3$); $[\alpha]_D$ +2.3 (*c* 3.5, EtOAc) (lit.[34] $[\alpha]_D$ +2.35 (*c* 3.5, EtOAc)); Anal. calcd. for $C_{27}H_{30}O_4$: C, 77.48; H, 7.22. Found: C, 77.19; H, 7.15%.

1D-2,3-Anhydro-4,5,6-tri-*O*-benzyl-*myo*-inositol (12)

m-CPBA (max 77% in H_2O, 1.89 g, 5.47 mmol) was added to a solution of cyclohexene **7R** (740 mg, 1.78 mmol) in CH_2Cl_2 (10 mL) under argon. The reaction was stirred under argon for 16 h after which time TLC (EtOAc–hexane, 1:2) showed that the reaction was finished. To the mixture was added CH_2Cl_2 (50 mL), 1 M aqueous Na_2SO_3 (30 mL), and saturated aqueous $NaHCO_3$ (30 mL). The phases were separated and the organic phase was washed carefully with saturated aqueous $NaHCO_3$ (30 mL). The combined aqueous phases were extracted with CH_2Cl_2 (25 mL). The combined organic phases were dried with Na_2SO_4 and concentrated. Purification by column chromatography (EtOAc–hexane, 2:3) gave 697 mg (91%) **12** as white crystals. R_f 0.32 (EtOAc–hexane, 2:3); mp 144–146 °C (EtOAc–hexane); $[\alpha]_D$ −57.1 (*c* 1.1, $CHCl_3$); δ_H (300 MHz, $CDCl_3$): 7.40–7.23 (m, 15H), 4.93 (d, *J* = 11.3 Hz, 1H), 4.82 (s, 2H), 4.78 (d, *J* = 11.3 Hz, 1H), 4.70 (d, *J* = 11.3 Hz, 1H), 4.62 (d, *J* = 11.3 Hz, 1H), 4.00 (dd, *J* = 8.2, 1.8 Hz, 1H), 3.92 (dd, *J* = 7.6, 0.7 Hz, 1H), 3.54–3.38 (m, 3H), 3.22 (d, *J* = 3.9 Hz, 1H), 2.40 (s, OH); δ_C (75 MHz, $CDCl_3$): 138.4, 138.3, 137.5, 128.7, 128.7, 128.5, 128.1, 128.1, 128.0, 127.9, 127.8, 83.4, 79.6, 79.4, 75.7, 75.4, 73.3, 72.0, 56.4, 53.7; Anal. calcd. for $C_{27}H_{28}O_5$: C, 74.98; H, 6.53. Found: C, 75.06; H, 6.45%.

1D-2,3-Anhydro-4,5,6-tri-*O*-benzyl-1-*O*-*p*-methoxybenzyl-*myo*-inositol (13)

NaH (50% dispersion in oil, 100 mg, 2.07 mmol) was added to a solution of **12** (600 mg, 1.38 mmol) in DMF (10 mL) under argon. The mixture was stirred for 40 min followed by addition of *p*-methoxybenzyl chloride (0.30 mL, 2.21 mmol). TLC (EtOAc–hexane, 1:4) showed that the reaction had gone to completion after 1.5 h at room temperature and the mixture was quenched with MeOH (2 mL). The solution was diluted with Et_2O (50 mL) and washed with water (2 × 25 mL) and brine (25 mL). The organic phase was dried with Na_2SO_4, concentrated, and purified by column chromatography (EtOAc–hexane, 1:3) to give 690 mg (90%) of **13** as white crystals. R_f 0.19 (EtOAc–hexane, 1:4); mp 101 °C (EtOAc–hexane); $[\alpha]_D$ −28.3 (*c* 1.3, $CHCl_3$); δ_H (300 MHz, $CDCl_3$): 7.37–7.17 (m, 17H), 6.87–6.82 (m, 2H), 4.86–4.66 (m, 8H), 3.89–3.85 (m, 2H), 3.76 (s, 3H), 3.61 (dd, *J* = 10.4, 8.5 Hz, 1H), 3.46 (dd, *J* = 10.4, 7.9 Hz, 1H), 3.27 (dd, *J* = 3.7, 1.7 Hz, 1H),

3.17 (d, J = 3.8 Hz, 1H); δ_C (75 MHz, $CDCl_3$): 159.3, 138.6, 138.5, 137.6, 130.3, 129.6, 128.5, 128.4, 128.3, 128.1, 128.0, 127.9, 127.8, 127.6, 113.8, 83.4, 79.2, 79.0, 79.0, 76.0, 75.6, 73.2, 72.8, 55.3, 55.3, 53.9; Anal. calcd. for $C_{35}H_{36}O_6$: C, 76.06; H, 6.57. Found: C, 75.98; H, 6.49%.

1D-4,5,6-Tri-*O*-benzyl-3-deoxy-1-*O*-*p*-methoxybenzyl-*myo*-inositol (14)

To a solution of epoxide **13** (495 mg, 0.90 mmol) in 1,2-dimethoxyethane (30 mL) under argon was added $NaBH_4$ (204 mg, 5.37 mmol). $BF_3{\cdot}OEt_2$ (0.283 mL, 2.24 mmol) was then added dropwise at a rate ensuring that the mixture remained alkaline. The reaction was stirred for 2 h after which time TLC (EtOAc–hexane, 1:3) showed no remaining starting material. The mixture was quenched with ice and neutralised by dropwise addition of 1 M aqueous HCl. Water (50 mL) was added and the solution was extracted with CH_2Cl_2 (3 × 25 mL). The combined organic phases were dried with $MgSO_4$ and concentrated. Purification by column chromatography (EtOAc–CH_2Cl_2–hexane, 1:4:5) gave 327 mg (66%) of the desired isomer **14** and 110 mg (22%) of the wrong regioisomer.

For **14**. R_f 0.18 (EtOAc–CH_2Cl_2–hexane, 1:4:5); $[\alpha]_D$ −8.60 (c 0.5, $CHCl_3$); δ_H (300 MHz, $CDCl_3$): 7.37–7.21 (m, 17H), 6.87–6.81 (m, 2H), 4.97–4.54 (m, 8H), 4.06 (dd, J = 3.0, 2.9 Hz, 1H), 3.99–3.88 (m, 1H), 3.81 (t, J = 9.4 Hz, 1H), 3.77 (s, 3H), 3.52–3.43 (m, 2H), 2.54 (s, OH), 2.35 (dt, J = 14.0, 4.2 Hz, 1H), 1.43–1.32 (m, 1H); δ_C (75 MHz, $CDCl_3$): 159.4, 139.0, 138.9, 138.8, 130.1, 129.6, 128.4, 128.0, 128.0, 127.8, 127.6, 127.6, 113.9, 85.8, 82.6, 81.7, 77.2, 76.1, 75.8, 73.0, 72.6, 66.0, 55.3, 32.6; Anal. calcd. for $C_{35}H_{38}O_6$: C, 75.79; H, 6.91. Found: C, 75.76; H, 6.88%.

1D-2,4,5,6-Tetra-*O*-benzyl-3-deoxy-1-*O*-*p*-methoxybenzyl-*myo*-inositol (15)

Alcohol **14** (260 mg) was treated with benzyl bromide (96 μL) as described above in the synthesis of **13** to afford 288 mg (95%) of **15** as a colourless oil. R_f 0.28 (Et_2O–CH_2Cl_2–hexane, 0.5:4.5:5); $[\alpha]_D$ +4.10 (c 1.1, $CHCl_3$); δ_H (300 MHz, $CDCl_3$): 7.39–7.19 (m, 22H), 6.86–6.80 (m, 2H), 4.96–4.81 (m, 4H), 4.68–4.48 (m, 6H), 4.02–3.75 (m, 6H), 3.48 (t, J = 9.2 Hz, 1H), 3.40 (dd, J = 9.7, 2.9 Hz, 1H), 2.18 (dt, J = 13.9, 4.4 Hz, 1H), 1.24–1.14 (m, 1H); δ_C (75 MHz, $CDCl_3$): 159.2, 139.1, 139.0, 138.8, 138.7, 130.7, 129.4, 128.4, 128.4, 128.2, 128.0, 128.0, 127.8, 127.7, 127.6, 127.6, 113.8, 86.2, 82.8, 82.1, 77.1, 76.1, 75.9, 73.0, 72.6, 72.4, 71.7, 55.3, 31.2; Anal. calcd. for $C_{42}H_{44}O_6$: C, 78.23; H, 6.88. Found: C, 78.43; H, 6.85%.

1D-2,4,5,6-Tetra-*O*-benzyl-3-deoxy-*myo*-inositol (5)

To a solution of **15** (229 mg, 0.36 mmol) in CH_2Cl_2 (15 mL) and H_2O (0.75 mL) was added DDQ (120 mg, 0.53 mmol). The reaction stirred for 2 h after which time TLC (EtOAc–CH_2Cl_2–hexane, 1:4:5) showed complete conversion. The mixture was filtered through Celite, concentrated and purified by column chromatography (EtOAc–CH_2Cl_2–hexane, 1:4:5) to give 160 mg (85%) of **5** as a colourless oil. R_f 0.20 (EtOAc–CH_2Cl_2–hexane, 1:4:5); $[\alpha]_D$ −7.79 (c 5.9, $CHCl_3$) (lit.[34] $[\alpha]_D$ −7.34 (c 0.62, $CHCl_3$)); Anal. calcd. for $C_{34}H_{36}O_5$: C, 77.84; H, 6.92. Found: C, 77.56; H, 6.89%.

1D-1-*O*-Benzoyl-3,4,5-tri-*O*-benzyl-*myo*-inositol (17B)

A solution of **7R** (390 mg, 0.94 mmol), DMAP (13 mg, 0.1 mmol), Et_3N (459 μL, 3.29 mmol), and benzoyl chloride (327 μL, 2.80 mmol) in dry CH_2Cl_2 (50 mL) was stirred under argon overnight. TLC (EtOAc–hexane, 1:3) showed full conversion. H_2O (5 mL) was added and the mixture stirred for another 30 min. The solution was diluted with CH_2Cl_2 (75 mL) and washed with H_2O (2 × 30 mL), dried with $MgSO_4$ and concentrated. The crude material was passed through a short silica column (8 cm) (Et_2O–hexane, 1:4) to give 570 mg of **16B** containing some benzoic anhydride, which could not be separated from the product. R_f 0.23 (Et_2O–hexane, 1:4). This material was dissolved in CH_2Cl_2 (40 mL), and NMO (178 mg, 1.3 mmol) and quinuclidine (10 mg) were added followed by 0.5 M aqueous OsO_4 (300 μL, 0.15 mmol). The yellow solution was stirred overnight after which time 1 M aqueous Na_2SO_3 (40 mL) was added. The aqueous phase was separated and extracted with CH_2Cl_2 (3 × 25 mL). The combined organic phases were washed with water (25 mL), dried with $MgSO_4$, concentrated, and purified by column chromatography (EtOAc–hexane, 2:5) to yield 450 mg (87% from **7R**) of **17B** as a white solid. R_f 0.25 (EtOAc–hexane, 2:3); $[\alpha]_D$ −32.1 (c 1.0, $CHCl_3$); δ_H (500 MHz, $CDCl_3$): 8.04 (d, J = 7.1 Hz, 2H), 7.54 (t, J = 7.4 Hz, 1H), 7.41 (t, J = 7.8 Hz, 2H), 7.34–7.08 (m, 15H), 5.12 (dd, J = 10.1, 2.7 Hz, 1H), 4.96 (d, J = 11.3 Hz, 1H), 4.91 (d, J = 10.9 Hz, 1H), 4.85 (d, J = 10.9 Hz, 1H), 4.81 (d, J = 10.9 Hz, 1H), 4.75 (d, J = 10.9 Hz, 1H), 4.73 (d, J = 11.3 Hz, 1H), 4.28 (t, J = 2.7 Hz, 1H), 4.21 (t, J = 9.5 Hz, 1H), 3.85 (t, J = 9.5 Hz, 1H), 3.64 (dd, J = 9.5, 2.5 Hz, 1H), 3.61 (t, J = 9.4 Hz, 1H), 2.63 (s, OH), 2.55 (s, OH); δ_C (125 MHz, $CDCl_3$): 165.9, 138.6, 138.5, 138.2, 133.4, 129.9, 129.9, 128.8, 128.6, 128.5, 128.4, 128.1, 128.0, 128.0, 127.8, 127.7, 83.5, 81.3, 79.8, 75.9, 75.9, 75.7, 74.1, 71.7, 70.1; Anal. calcd. for $C_{34}H_{34}O_7$: C, 73.63; H, 6.18. Found: C, 73.56; H, 6.22%.

1D-1-*O*-Benzoyl-2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol (18)

Benzyl trichloroacetimidate (606 mg, 2.40 mmol) was added to a solution of **17B** (220 mg, 0.40 mmol) in dioxane (6 mL) under argon. Triflic acid was added until the solution became strongly acidic (~15 drops). The mixture was stirred for 30 min after which time TLC (EtOAc–hexane, 1:4) showed that the reaction had gone to completion. The solution was diluted with Et_2O (30 mL) and washed with saturated aqueous $NaHCO_3$ (2 × 15 mL) and water (2 × 15 mL). The organic phase was dried with Na_2SO_4, concentrated, and purified by column chromatography (Et_2O–CH_2Cl_2–hexane, 0.5:4.5:5.5) to give 247 mg (85%) of **18** as a white solid. R_f 0.46 (EtOAc–hexane, 1:4); mp 106–108 °C (Et_2O–hexane); $[\alpha]_D$ −2.1 (c 1.0, $CHCl_3$); δ_H (500 MHz, $CDCl_3$): 7.99 (d, J = 7.1 Hz, 2H), 7.55 (t, J = 7.3 Hz, 1H), 7.41 (t, J = 7.8 Hz, 2H), 7.36–7.05 (m, 25H), 5.06 (dd, J = 10.3, 2.6 Hz, 1H), 4.98–4.62 (m, 10H), 4.30–4.23 (m, 2H), 4.15 (t, J = 9.6 Hz, 1H), 3.64–3.55 (m, 2H); δ_C (125 MHz, $CDCl_3$): 166.0, 138.9, 138.8, 138.6, 138.4, 138.3, 133.3, 129.9, 128.6, 128.5, 128.5, 128.3, 128.3, 128.1, 128.0, 127.9, 127.8, 127.6, 83.7, 81.7, 81.1, 79.8, 76.1, 76.0, 75.8, 75.4, 74.9, 74.5, 73.1.

1D-2,3,4,5,6-Penta-*O*-benzyl-*myo*-inositol (6)

Benzoate **18** (220 mg, 0.30 mmol) was dissolved in 0.45 M MeONa in MeOH (30 mL) and stirred for 1.5 h. TLC (EtOAc–hexane, 1:4) showed full conversion and the reaction was quenched with Amberlite IR-120 (H^+) ion exchange resin (20 mL). The mixture was filtered and concentrated. Purification by column chromatography (EtOAc–hexane, 1:4) gave 178 mg (95%) of **6** as a white solid. R_f 0.20 (EtOAc–hexane, 1:4); $[\alpha]_D$ −9.0 (c 1.0, $CHCl_3$) (lit.[35] $[\alpha]^{20}_D$ −9.0 (c 1, $CHCl_3$)); Anal. calcd. for $C_{41}H_{42}O_6$: C, 78.07; H, 6.71. Found: C, 78.28; H, 6.78%.

1D-2,3-Dideoxy-*myo*-inositol 1-[(*R*)-3-(hexadecyloxy)-2-hydroxypropyl hydrogen phosphate] (1)

To a solution of **4** (200 mg, 0.48 mmol) in CH_2Cl_2 (8 mL) under argon was added 2,2,6,6-tetramethylpiperidine (115 mg, 0.82 mmol) followed by benzyl-*N*,*N*-diisopropylchlorophosphoramidite (151 mg, 0.77 mmol). The solution was stirred for 1 h after which time TLC indicated that the reaction had gone to completion (EtOAc–hexane, 1:3 with 5% Et_3N, TLC plates were neutralised in Et_3N prior to use). The reaction was quenched by the addition of saturated aqueous $NaHCO_3$ (25 mL) and CH_2Cl_2 (25 mL), and the phases were separated. The organic phase was washed with saturated aqueous $NaHCO_3$

(2 × 25 mL), dried with Na_2SO_4, and concentrated. The crude product was co-evaporated twice with toluene and then dissolved in CH_2Cl_2 (10 mL) followed by addition of (*S*)-1-*O*-hexadecyl-2-*O*-benzyl-glycerol (**19**)[29] (283 mg, 0.70 mmol) and 4 Å molecular sieves. The suspension was stirred for 40 min after which time the mixture was cooled to 0 °C and 5-phenyl-1*H*-tetrazole (105 mg, 0.72 mmol) was added. The suspension was stirred for 1.5 h and allowed to warm to room temperature. It was then cooled to 0 °C again and 5.5 M *t*-BuOOH in dry hexane (130 µL, 0.72 mmol) was added. The mixture was stirred for 1 h and then quenched by the addition of 1 M aqueous Na_2SO_3 (20 mL) and saturated aqueous $NaHCO_3$ (20 mL). The phases were separated after 15 min stirring and the aqueous phase was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic phases were washed with brine (25 mL), dried with $MgSO_4$, and concentrated. Purification by column chromatography (EtOAc–hexane, 3 : 7, R_f 0.22) gave the benzyl protected phosphatidylinositol as a mixture of two diastereomers. These were dissolved in *t*-BuOH (20 mL) and 10% Pd/C (70 mg) was added. The suspension was hydrogenated under 10 bar of H_2 for 18 h, and then filtered and concentrated to give 176 mg (70%) of **1**. R_f 0.20 ($CHCl_3$–MeOH–H_2O, 65 : 25 : 4); δ_H (500 MHz, $CDCl_3$–CD_3OD, 9 : 1): 4.15–3.98 (m, 4H), 3.50–3.40 (m, 5H), 3.39 (t, J = 9.1 Hz, 1H), 3.24 (t, J = 9.1 Hz, 1H), 2.12 (m, 1H), 1.94 (m, 1H), 1.59–1.48 (m, 3H), 1.37 (m, 1H), 1.36–1.27 (br s, 26H), 0.89 (t, J = 6.8 Hz, 3H); δ_C (75 MHz, $CDCl_3$–CD_3OD, 9 : 1): 79.0 (J = 6.2 Hz), 77.2, 75.8 (J = 5.6 Hz), 71.8, 71.7, 71.0, 69.1 (J = 7.3 Hz), 68.6 (J = 6.2 Hz), 31.8, 29.6, 29.5, 29.4, 29.2, 29.2, 27.6, 27.3 (J = 1.3 Hz), 25.9, 22.6, 13.9; δ_P (202 MHz, $CDCl_3$–CD_3OD, 9 : 1): −0.21; ESI HRMS calcd. for $C_{25}H_{50}O_9P$ $[M - H]^-$ m/z 525.3192, found m/z 525.3148.

1D-3-Deoxy-*myo*-inositol 1-[(*R*)-3-(hexadecyloxy)-2-hydroxypropyl hydrogen phosphate] (2)

Phosphorylation of **5** (114 mg) was performed as described above to give 79 mg (67%) of **2**. R_f 0.17 ($CHCl_3$–MeOH–H_2O, 65 : 25 : 4); δ_H (500 MHz, $CDCl_3$–CD_3OD, 3 : 1): 4.24–4.21 (m, 1H), 4.10–3.94 (m, 5H), 3.87–3.75 (m, 3H), 3.58 (t, 9.2 Hz, 1H), 3.27–3.17 (m, 2H), 2.16–2.10 (m, 1H), 1.61–1.23 (br s, 29H), 0.89 (t, J = 6.8 Hz, 3H); δ_C (75 MHz, $CDCl_3$–CD_3OD, 3 : 1): 79.6 (J = 5.6 Hz), 77.4 (J = 3.6 Hz), 73.9, 72.5, 71.3, 70.9, 69.0 (J = 7.1 Hz), 67.9 (J = 4.4 Hz), 67.4 (J = 5.1 Hz), 31.4, 29.2, 29.1, 29.0, 29.0, 28.8, 25.5, 22.1, 13.2; δ_P (202 MHz, $CDCl_3$–CD_3OD, 3 : 1): 1.18; ESI HRMS calcd. for $C_{25}H_{50}O_{10}P$ $[M - H]^-$ m/z 541.3141, found m/z 541.3088.

1D-*myo*-Inositol 1-[(*R*)-3-(hexadecyloxy)-2-hydroxypropyl hydrogen phosphate] (3)

Phosphorylation of **6** (158 mg) was performed as described above to give 92 mg (66%) of **3**. R_f 0.14 ($CHCl_3$–MeOH–H_2O, 65 : 25 : 4); δ_H (300 MHz, $CDCl_3$–CD_3OD, 1 : 1): 4.25–4.19 (m, 1H), 4.09–3.90 (m, 4H), 3.80 (t, J = 9.2 Hz, 1H), 3.66 (t, J = 9.5 Hz, 1H), 3.53–3.37 (m, 5H), 3.23 (t, J = 9.2 Hz, 1H), 1.64–1.52 (m, 2H), 1.34–1.20 (br s, 26H), 0.89 (t, J = 6.6 Hz, 3H); δ_C (75 MHz, $CDCl_3$–CD_3OD–D_2O, 65 : 25 : 4): 77.3, 76.2, 73.9, 72.1, 71.6, 71.3, 71.1, 70.7, 69.3, 67.1, 31.7, 29.5, 29.4, 29.4, 29.2, 29.1, 25.7, 22.4, 13.7; δ_P (202 MHz, $CDCl_3$–CD_3OD, 1 : 1): 0.05; ESI HRMS calcd. for $C_{25}H_{50}O_{11}P$ $[M - H]^-$ m/z 557.3091, found m/z 557.3079.

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References

1 C. Pendaries, H. Tronchére, M. Plantavid and B. Payrastre, *FEBS Lett.*, 2003, **546**, 25.
2 B. V. L. Potter and D. Lampe, *Angew. Chem., Int. Ed. Engl.*, 1995, **34**, 1933.
3 M. J. Berridge and R. F. Irvine, *Nature*, 1989, **341**, 197.
4 M. J. Berridge, *Nature*, 1993, **361**, 315.
5 A. M. Shamsuddin, I. Vucenik and K. E. Cole, *Life Sci.*, 1997, **61**, 343.
6 L. Qiao, F. Nan, M. Kunkel, A. Gallegos, G. Powis and A. P. Kozikowski, *J. Med. Chem.*, 1998, **41**, 3303.
7 T. G. Cross, D. Scheel-Toellner, N. V. Henriquez, E. Deacon, M. Salmon and J. M. Lord, *Exp. Cell Res.*, 2000, **256**, 34.
8 I. Vivanco and C. L. Sawyers, *Nat. Rev. Cancer*, 2002, **2**, 489.
9 L. C. Cantley and B. G. Neel, *Proc. Natl. Acad. Sci. U. S. A.*, 1999, **96**, 4240.
10 (*a*) A. P. Kozikowski, H. Y. Sun, J. Brognard and P. A. Dennis, *J. Am. Chem. Soc.*, 2003, **125**, 1144; (*b*) E. J. Meuillet, D. Mahadevan, H. Vankayalapati, M. Berggren, R. Williams, A. Coon, A. P. Kozikowski and G. Powis, *Mol. Cancer Ther.*, 2003, **2**, 389.
11 M. Lohmeyer and R. Bittman, *Drugs Future*, 1994, **19**, 1021.
12 G. A. Ruiter, S. F. Zerp, H. Bartelink, W. J. van Blitterswijk and M. Verheij, *Anti-Cancer Drugs*, 2003, **14**, 167.
13 I. Ahmad, J. J. Filep, J. C. Franklin, A. S. Janoff, G. R. Masters, J. Pattassery, A. Peters, J. J. Schupsky, Y. Zha and E. Mayhew, *Cancer Res.*, 1997, **57**, 1915.
14 (*a*) T. L. Andresen, J. Davidsen, M. Begtrup, O. G. Mouritsen and K. Jørgensen, *J. Med. Chem.*, 2004, **47**, 1694; (*b*) J. Davidsen, K. Jørgensen, T. L. Andresen and O. G. Mouritsen, *Biochim. Biophys. Acta*, 2003, **1609**, 95.
15 (*a*) K. M. Sureshan, T. Yamasaki, M. Hayashi and Y. Watanabe, *Tetrahedron: Asymmetry*, 2003, **13**, 1771; (*b*) G. F. Painter, S. J. A. Grove, I. H. Gilbert, A. B. Holmes, P. R. Raithby, M. L. Hill, P. T. Hawkins and L. R. Stephens, *J. Chem. Soc., Perkin Trans. 1*, 1999, 923; (*c*) L. W. Leung and R. Bittman, *Carbohydr. Res.*, 1998, **305**, 171; (*d*) R. Madsen, U. E. Udodong, C. Roberts, D. R. Mootoo, P. Konradsson and B. Fraser-Reid, *J. Am. Chem. Soc.*, 1995, **117**, 1554; (*e*) L. Ling and S. Ozaki, *Carbohydr. Res.*, 1994, **256**, 49; (*f*) K. S. Bruzik and M.-D. Tsai, *J. Am. Chem. Soc.*, 1992, **114**, 6361; (*g*) G. R. Baker, D. C. Billington and D. Gani, *Tetrahedron*, 1991, **47**, 3895; (*h*) C. J. J. Elie, R. Verduyn, C. E. Dreef, D. M. Brounts, G. A. van der Marel and J. H. van Boom, *Tetrahedron*, 1990, **46**, 8243; (*i*) J. P. Vacca, S. J. deSolms, J. R. Huff, D. C. Billington, R. Baker, J. J. Kulagowski and I. M. Mawer, *Tetrahedron*, 1989, **45**, 5679.
16 (*a*) B. R. Sculimbrene, A. J. Morgan and S. J. Miller, *J. Am. Chem. Soc.*, 2002, **124**, 11653; (*b*) B. M. Trost, D. E. Patterson and E. J. Hembre, *J. Am. Chem. Soc.*, 1999, **121**, 10834; (*c*) P. Andersch and M. P. Schneider, *Tetrahedron: Asymmetry*, 1993, **4**, 2135.
17 (*a*) R. M. Conrad, M. J. Grogan and C. R. Bertozzi, *Org. Lett.*, 2002, **8**, 1359; (*b*) A. Nishikawa, S. Saito, Y. Hashimoto, K. Koga and R. Shirai, *Tetrahedron Lett.*, 2001, **42**, 9195; (*c*) H. Takahashi, H. Kittaka and S. Ikegami, *J. Org. Chem.*, 2001, **66**, 2705; (*d*) J. Chen, L. Feng and G. D. Prestwich, *J. Org. Chem.*, 1998, **63**, 6511; (*e*) A. Kornienko, D. I. Turner, C. H. Jaworek and M. d'Alarcao, *Tetrahedron: Asymmetry*, 1998, **9**, 2783; (*f*) J. L. Chiara and M. Martín-Lomas, *Tetrahedron Lett.*, 1994, **35**, 2969.
18 For recent reviews, see: (*a*) A. Deiters and S. F. Martin, *Chem. Rev.*, 2004, **104**, 2199; (*b*) R. R. Schrock and A. H. Hoveyda, *Angew. Chem. Int. Ed.*, 2003, **42**, 4592; (*c*) S. Blechert and S. J. Connon, *Angew. Chem. Int. Ed.*, 2003, **42**, 1900; (*d*) C. S. Poulsen and R. Madsen, *Synthesis*, 2003, 1; (*e*) T. M. Trnka and R. H. Grubbs, *Acc. Chem. Res.*, 2001, **34**, 18; (*f*) A. Fürstner, *Angew. Chem. Int. Ed.*, 2000, **39**, 3013.
19 (*a*) C. S. Poulsen and R. Madsen, *J. Org. Chem.*, 2002, **67**, 4441; (*b*) M. Jørgensen, E. H. Iversen, A. L. Paulsen and R. Madsen, *J. Org. Chem.*, 2001, **66**, 4630; (*c*) L. Hyldtoft and R. Madsen, *J. Am. Chem. Soc.*, 2000, **122**, 8444.
20 For a review, see: M. Jørgensen, P. Hadwiger, R. Madsen, A. E. Stütz and T. M. Wrodnigg, *Curr. Org. Chem.*, 2000, **4**, 565.
21 P. R. Skaanderup and R. Madsen, *J. Org. Chem.*, 2003, **68**, 2115.
22 P. R. Skaanderup, C. S. Poulsen, L. Hyldtoft, M. R. Jørgensen and R. Madsen, *Synthesis*, 2002, 1721.
23 (*a*) P. R. Skaanderup, L. Hyldtoft and R. Madsen, *Monatsh. Chem.*, 2002, **133**, 467; (*b*) B. Bernet and A. Vasella, *Helv. Chim. Acta*, 1979, **62**, 1990.
24 A. Boschetti, F. Nicotra, L. Panza and G. Russo, *J. Org. Chem.*, 1988, **53**, 4181.
25 A. Kornienko and M. d'Alarcao, *Tetrahedron: Asymmetry*, 1999, **10**, 827.
26 C. Jaramillo, J.-L. Chiara and M. Martín-Lomas, *J. Org. Chem.*, 1994, **59**, 3135.
27 T. Trnka, M. Budešínský and M. Cerný, *Carbohydr. Res.*, 1994, **259**, 131.
28 O. Arjona, A. de Dios, J. Plumet and B. Saez, *J. Org. Chem.*, 1995, **60**, 4932.

29 (*a*) U. Massing and H. Eibl, *Chem. Phys. Lipids*, 1995, **76**, 221; (*b*) F. Bauer, K.-P. Rueß and M. Liefländer, *Liebigs Ann. Chem.*, 1992, 47; (*c*) P. N. Guivisdalsky and R. Bittman, *J. Org. Chem.*, 1989, **54**, 4643.
30 S. L. Beaucage and R. P. Iyer, *Tetrahedron*, 1993, **49**, 10441.
31 Purchased from Digital Specialty Chemicals Ltd.
32 1*H*-Tetrazole is the standard proton source in the phosphoramidite coupling. However, most chemical vendors do not supply 1*H*-tetrazole as a crystalline compound any more, but instead a saturated acetonitrile solution is offered. Although, we had no difficulties in using this acetonitrile solution, we found the crystalline 5-phenyl-1*H*-tetrazole to be a convenient alternative that gave the same coupling yields.
33 B. M. Trost, D. E. Patterson and E. J. Hembre, *Chem. Eur. J.*, 2001, **7**, 3768.
34 A. P. Kozikowski, L. Qiao, W. Tückmantel and G. Powis, *Tetrahedron*, 1997, **53**, 14903.
35 C. E. Dreef, M. Douwes, C. J. J. Elie, G. A. van der Marel and J. H. van Boom, *Synthesis*, 1991, 443.