Organic & Biomolecular Chemistry

PAPER

View Article Online
View Journal | View Issue

Cite this: *Org. Biomol. Chem.*, 2014, **12**, 1163

Synthesis of non-hydrolysable mimics of glycosylphosphatidylinositol (GPI) anchors†

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Received 24th October 2013,
Accepted 3rd December 2013

DOI: 10.1039/c3ob42116c

www.rsc.org/obc

Synthesis of first generation non-hydrolysable C-phosphonate GPI analogs, *viz.*, 6-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-D-*myo*-inositol-1-*O*-(*sn*-3,4-bis(palmitoyloxy)butyl-1-phosphonate) **23a** and 6-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-D-*myo*-inositol-1-*O*-(*sn*-2,3-bis(palmitoyloxy)propyl-1-phosphonate) **23b**, is reported. The target compounds were synthesized by the coupling of α-pseudodisaccharide **21** with phosphonic acids **18a** and **18b** respectively in quantitative yield followed by deprotection. These synthetic C-phosphonate GPI-probes were resistant to phosphatidylinositol specific phospholipase C (PI-PLC) and also showed moderate inhibition of the enzyme activity.

Glycosylphosphatidylinositols (GPIs) are a unique class of natural glycophospholipids which provide an alternative mechanism for anchoring of a number of specialized proteins to the outer leaflet of the eukaryotic plasma membrane.[1–3] The GPI-anchored proteins (GPI-APs) along with cholesterol and sphingolipids create functional nanodomains (lipid rafts) which are in turn enriched in specific regions of the cell by a mechanism that involves dynamic actin activity.[4] These nano-domain enriched regions are likely to act as signaling platforms at the cell surface.[5] The understanding of nano-domain enriched regions has provided a new functional dynamic view of the plasma membrane generating significant interest in cell and membrane biology as to how the GPI anchored proteins (GPI-APs) interact with cholesterol leading to the formation of ordered domains in biological membranes.[6,7] The molecular understanding of this mechanism of the localized clustering of GPI-anchored proteins[4] and cholesterol in such domains requires synthetic and metabolically stable GPI probes.

Due to a very short half-life in biological systems, GPI anchors are cleaved by specific phospholipases (GPI-specific phospholipase C and PI-specific phospholipase C)[8] at the phosphodiester linkage with the assistance of the nucleophilic 2-OH of D-*myo*-inositol. In some instances this cleavage releases important second messengers such as diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP_3) which activate numerous important cellular processes such as DNA synthesis, cell proliferation and neuronal activity.[9] In this direction, the synthesis of non-hydrolysable PLC-resistant synthetic GPI probes will greatly help in deciphering the role of the GPI-head group in the formation of signaling platforms or functional domains.[10] A variety of non-hydrolysable PLC-resistant PI substrates have been synthesized following several approaches, *viz.*, modification of nucleophilic the 2-OH of inositol,[11] replacement of the scissile P–O bond with a C–P bond[12] and conformationally constrained analogs.[13] Only one report towards the synthesis of a non-hydrolysable PLC resistant GPI analog was reported by Morris *et al.*, in which GPI analogs, *viz.* glucosaminyl(α1-6)-D-*myo*-inositol-1-acyl-phosphonates, were used to study the role of glycan for recognition by GPI-PLC;[8a] however, the synthesis of this analog was never published. Our continued interest in the chemistry and biology of GPI molecules[4,14] motivated us to initiate efforts towards synthesis of non-hydrolysable PLC resistant GPI analogs where normal scissile P–O bond of the phosphodiester linkage was replaced with a C–P bond.

We now report a new synthetic strategy for preparation of first generation non-hydrolysable PLC resistant GPI analogs **23a** and **23b**, and demonstrate that the C–P containing GPI anchors were not hydrolyzed by PI-PLC.

Our approach to the synthesis of non-hydrolysable GPI anchors **23a** and **23b** is outlined in Scheme 1, and comprises three main stages: (i) synthesis of suitably protected glucosaminyl(α1-6)-D-*myo*-inositol **21**; (ii) synthesis of optically pure

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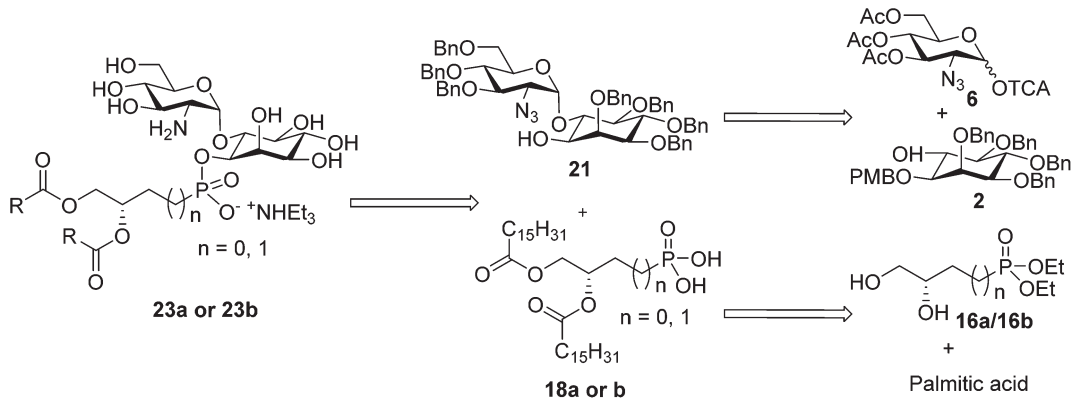
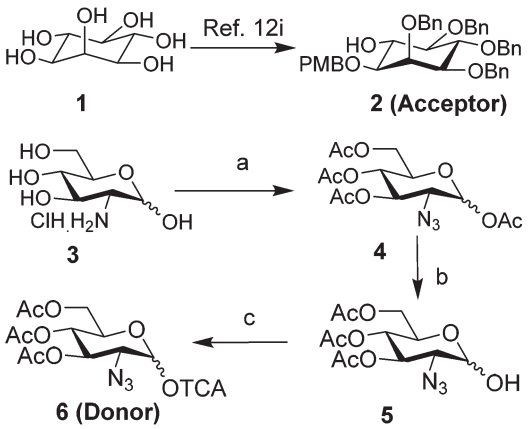
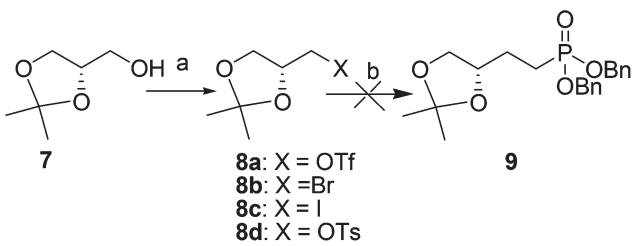
†Electronic supplementary information (ESI) available: ^1H and ^{13}C NMR and HRMS spectra of all compounds and the details of inhibition experiments. See DOI: 10.1039/c3ob42116c



Scheme 1 Retro-synthetic overview.



Scheme 2 Reagents and conditions: (a) i. TfN_3, H_2O, K_2CO_3, CH_2Cl_2, MeOH, $CuSO_4$, −10 °C to rt, 24 h; ii. Ac_2O, pyridine, DMAP, 24 h, 80% over 2 steps; (b) hydrazine acetate, DMF, 0 °C to rt, 1 h, 85%; (c) Cl_3CCN, CH_2Cl_2, K_2CO_3, rt, 2 h, 84%.



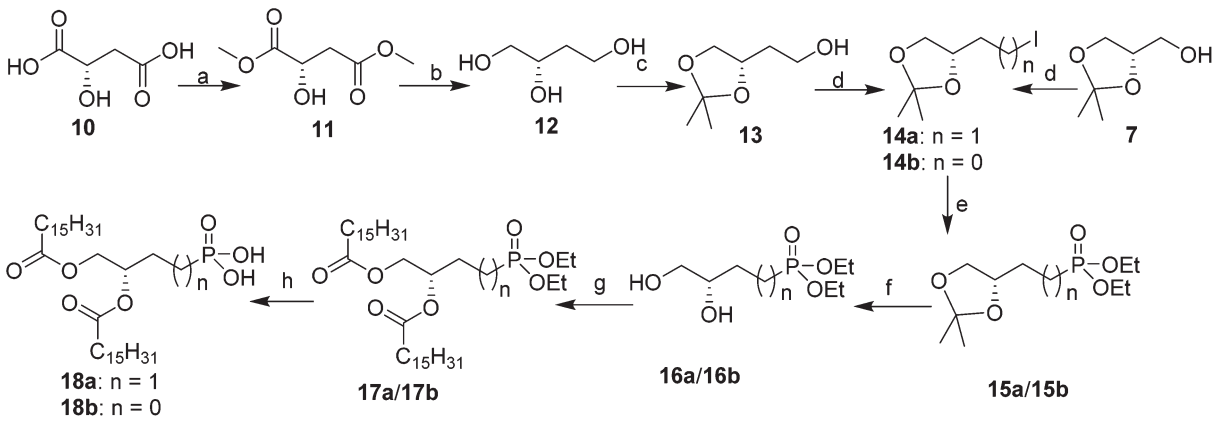
Scheme 3 Reagents and conditions: (a) Tf_2O, TEA, CH_2Cl_2, −20 °C, 1 h, 98% for **8a**; PPh_3, CBr_4, CH_2Cl_2, 0 °C then rt, 3 h, 99% for **8b**; PPh_3, I_2, imidazole, toluene, 90 °C, 2 h, 95% for **8c**; *p*-TsCl, pyridine, rt, 24 h, 70% for **8d**; (b) $CH_3P(OBn)_2$, base, THF, −78 °C, 2 h.

phosphonic acid donors **18a** and **18b**; and (iii) coupling of pseudo-disaccharide **21** with phosphonic acid donors **18a** and **18b** respectively.

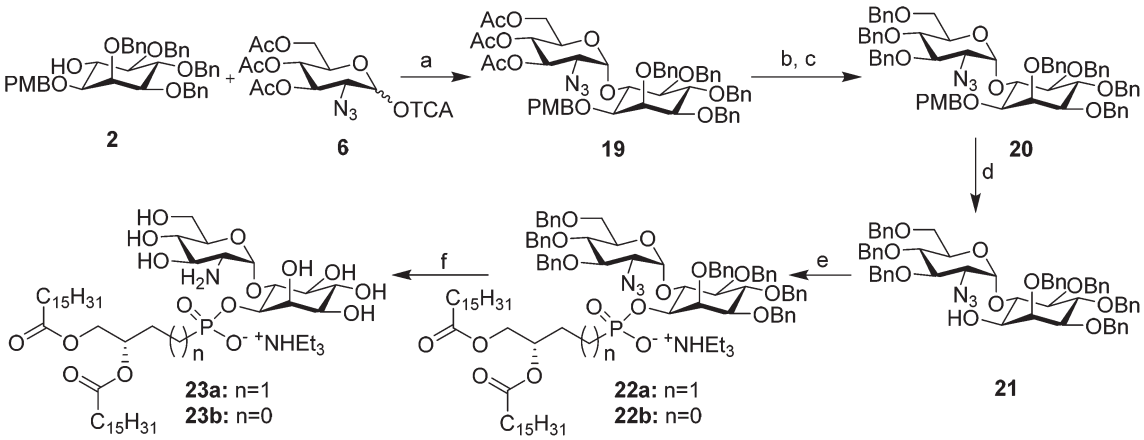
First, the synthesis of optically pure 1-*O*-*p*-methoxybenzyl-2,3,4,5-tetra-*O*-benzyl-D-*myo*-inositol **2** (Scheme 2, detail given in ESI†) was successfully achieved as per our recently published method.[14i] The 2-azido glycosyl donor **6** was synthesized from D-glucosamine **3** (Scheme 2) which on treatment with triflic azide followed by per acetylation gave 2-azido-2-deoxy-1,3,4,6-tetra-*O*-acetyl-D-glucosamine (**4**). The anomeric acetyl group of peracetylated compound **4** was selectively deprotected by hydrazine acetate at 0 °C, which on treatment with trichloroacetonitrile in the presence of potassium carbonate gave 2-azido-glycosyl trichloroacetimide donor **6** (α : β).

The synthesis of 2-deoxyerythrityl based C-phosphonic acid *i.e.*, 3,4-di-*O*-palmitoyloxybutylphosphonate **18a** was initially visualized as per the strategy shown in Scheme 3. The 2,3-isopropylidene glycerol **7** was first converted into triflate **8a** by treating with triflic anhydride. The coupling of triflate **8a** with dibenzyl methylphosphonate under various basic conditions (*n*-BuLi, LDA, LiHMDS at −78 °C and −40 °C) did not give the desired product **9**. Similarly, bromo **8b**, iodo **8c**, tosylate **8d** were also prepared from 2,3-isopropylidene glycerol **7** and tried coupling with dibenzyl methylphosphonate. However in this case the desired nucleophilic substitution reactions were not successful in our case, as previously reported in the literature.[15]

Later, a different approach for the synthesis of 3,4-di-*O*-palmitoyloxybutylphosphonate **18a** was tested out starting from optically pure *S*-(−)-malic acid **10** as given in Scheme 4. The *S*-(−)-malic acid was converted into *S*-(−)-dimethyl malate **11** which was then reduced with $LiBH_4$ and gave *S*-(−)-2-deoxy-erythritol **12**. The *S*-(−)-erythritol **12** on treatment with acetone in the presence of *p*-TSA gave a regioselective five-membered *S*-(−)-1,2-*O*-isopropylidene product **13**.[16] Compound **13** was converted into an iodo compound **14a** followed by treatment with triethylphosphite which gave *S*-(−)-diethyl phosphonate **15a**. Since *S*-(−)-diethyl phosphonate (**15a**) had the same polarity as triethyl phosphite or ethyl diethylphosphonate, the chromatographic separation became very cumbersome. Therefore, the isopropylidene protection was removed and the product *S*-(−)-diethyl 3,4-dihydroxybutylphosphonate (**16a**) could be easily purified by column chromatography. Further, diethyl 3,4-dihydroxybutylphosphonate (**16a**) when treated with palmitic acid in the presence of DCC gave *S*-(−)-diethyl 3,4-di-*O*-palmitoyloxybutyl-1-phosphonate (**17a**). The compound **17a** on hydrolysis in the presence of TMSBr gave 2,3-di-*O*-palmitoyloxybutyl-1-phosphonic acid (**18a**). Similarly, glyceryl based



Scheme 4 Reagents and conditions: (a) MeOH, PTSA, rt, 12 h, 86%; (b) $LiBH_4$, THF, rt then reflux, 2 h, 95%; (c) acetone, PTSA, reflux, 2 h, 87%; (d) PPh_3, I_2, imidazole, toluene, 90 °C, 2 h 98%; (e) $P(OEt)_3$, reflux, 6 h; (f) MeOH, PTSA, rt, 12 h, 82% over two steps; (g) palmitic acid, DCC, DMAP, CH_2Cl_2, rt, 12 h, 88%; (h) TMSBr, CH_2Cl_2, rt, 6 h then THF–H_2O, reflux, 1 h, 98%.



Scheme 5 Reagents and conditions: (a) TMSOTf, CH_2Cl_2, MS 4 Å, –78 °C, 2 h, 90%; (b) NaOMe, MeOH–CH_2Cl_2, rt, 8 h; (c) BnBr, DMF, NaH, rt, 3 h, 80% over two steps; (d) DDQ, H_2O, 0 °C to rt, 3 h, 55%; (e) **18a** or **18b**, Cl_3CCN, pyridine, 60 °C, 48 h, 77% for **22a** and 50% for **22b**; (f) 10% $Pd(OH)_2$/C, H_2, CH_2Cl_2–MeOH–H_2O, rt, 24 h, 80%.

C-phosphonic acid *i.e.*, 2,3-di-*O*-palmitoyloxypropyl-1-phosphonic acid (**18b**) was prepared from isopropylidene protected glycerol **7** following the same sequence of steps (Scheme 4) as discussed above.

Further, the inositol acceptor *i.e.*, 1-*O*-4-methoxybenzyl-2,3,4,5-tetra-*O*-benzyl-D-*myo*-inositol (**2**) was coupled with 2-azido glycosyl donor **6** in the presence of catalytic TMSOTf at −78 °C resulting in formation of the α-pseudodisaccharide **19** in excellent yield (Scheme 5).[14i] The acetyl group of the glycosyl moiety of α-pseudodisaccharide **19** was replaced with a benzyl protecting group (**19–20**). The PMB group was then removed (**20→21**) and the resulting free 1-OH was successfully coupled with *S*-(−)-3,4-di-*O*-palmitoyloxybutylphosphonic acid (**18a**) to produce fully protected C-phosphonates containing the GPI intermediate **22a**.[16] The compound **22a** was finally subjected to global benzyl deprotection and 2-azido reduction by Pearlman's hydrogenolysis method [$Pd(OH)_2$, CH_2Cl_2–MeOH–H_2O, H_2], which provided 6-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-D-*myo*-inositol-1-*O*-((*sn*-3,4-bis(palmitoyloxy)butyl-1-phosphonate) (**23a**). An identical approach was utilized to synthesize 6-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-D-*myo*-inositol-1-*O*-(*sn*-2,3-bis(palmitoyloxy)propyl-1-phosphonate) (**23b**) from its respective intermediates **21** and **18b**.

To determine the stability of synthetic C-phosphonates containing GPI anchors **23a** and **23b**, these analogs were subjected to PI-PLC (phosphatidylinositol-specific phospholipase C) enzymatic reaction[17] on a small scale. The enzymes PI-PLC and GPI-PLC are known to cleave the PI (phosphatidylinositol) as well as GPI substrates at the *sn*-3 phosphodiester bond.[8b,18] We chose fluorophore tagged PI (**24**, *sn*-2-NBD labeled PI) as a control substrate for the PI-PLC reaction; the substrate NBD labeled PI (**24**) was synthesized by a method reported by us.[14c] Since the *sn*-2 position of PI **24** was tagged to the NBD fluorophore, the enzymatic reaction could be easily monitored by TLC and MS. The NBD-PI **24** as well as C-phosphonate GPI probes **23a** and **23b** were treated with PI-PLC[19] for 20 minutes at 37 °C and checked for the completion of the reaction by TLC and MALDI-MS analysis. As evident from the TLC and

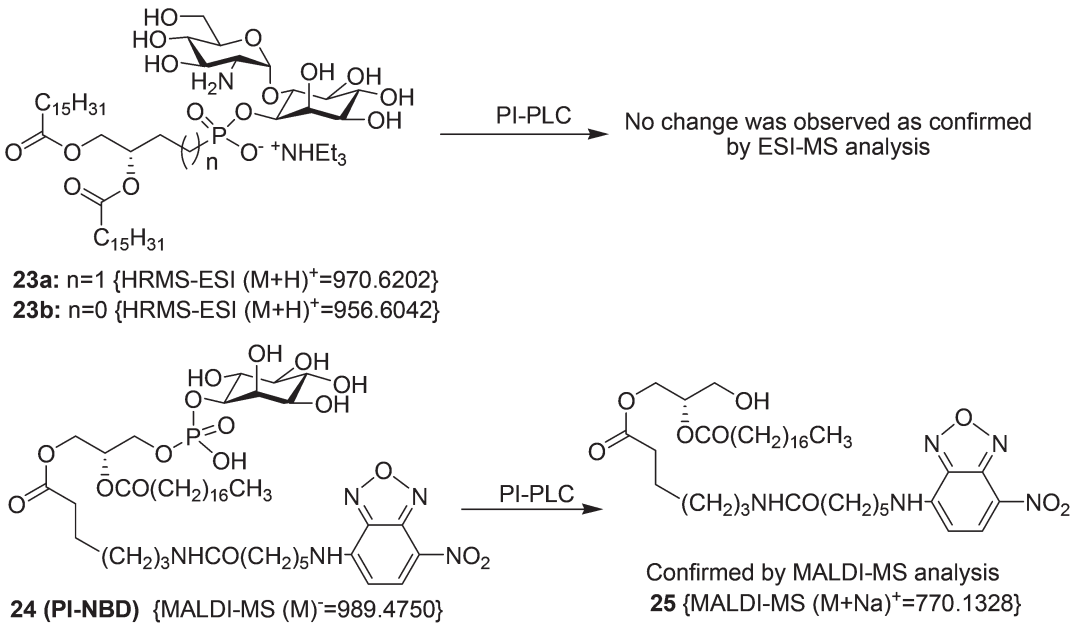


Fig. 1 PI-PLC enzymatic reaction of C-phosphonate GPI analogs **23a** and **23b** along with the standard PI-NBD **24**.

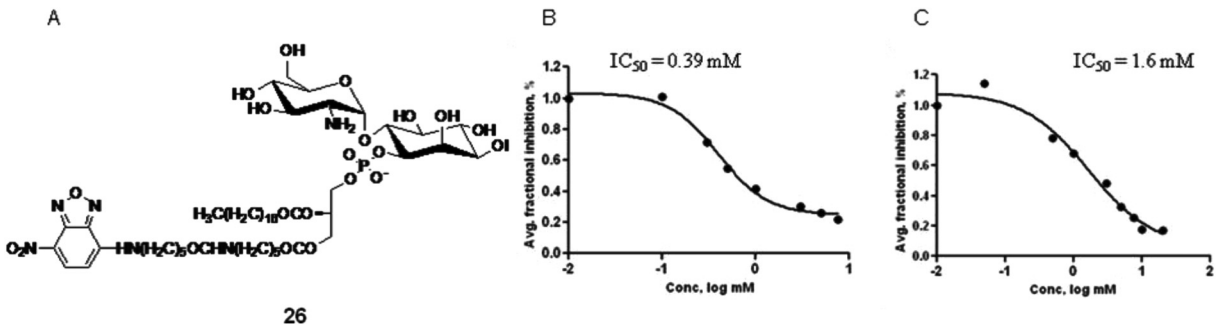


Fig. 2 (A) Represents the structure of NBD-GPI probes **26**. (B and C) Represent the IC_{50} of **23a** and **23b** for the PI-PLC inhibition activity.

mass analysis, NBD-PI **24** underwent complete hydrolysis (peak at 770.1328 observed corresponding to the hydrolyzed fragment **25**, Fig. 1) whereas the C-phosphonate GPI analogues did not get hydrolyzed (peaks at 970.6192 and 956.6042 corresponding to **23a** and **23b** respectively, Fig. 1).

Since both the non-hydrolysable C-phosphonate GPI probes **23a**/**23b** were substrate mimics of PI-PLC reaction, it was important to see if they also inhibited the enzyme. For this, we carried out inhibition experiments with the GPI probes **23a** and **23b** which required a synthetic fluorescent labeled enzyme substrate. We decided to use a NBD-labeled GPI analog **26** (Fig. 2A) as the substrate and synthesized it by a synthetic method designed[14b] by us earlier to address the mechanism of flip-flop of GPIs across the endoplasmic reticulum (ER). For inhibition experiments, various concentrations of **23a** and **23b** (7.5 mM to 0.1 mM) were added to the enzyme mix and the corresponding percentage cleavages of NBD-labeled GPI substrate **26** were calculated (TLC) which were then subtracted from the control reaction of the NBD-GPI substrate (without inhibitors) to calculate the percentage of inhibition. The IC_{50} values of 0.39 mM and 1.6 mM were obtained for **23a** and **23b** respectively (Fig. 2), confirming that these GPI probes moderately inhibited the PI-PLC activity (details of the experiments are given in Fig. 3 and 4 of ESI†).

In conclusion, we synthesized the first generation C-phosphonate containing GPI probes **23a** and **23b** and demonstrated that these synthetic probes are resistant to hydrolysis by PI-PLC and inhibited the enzyme moderately at high concentration, the attributes making them suitable for cell biology experiments. Efforts towards synthesizing advanced membrane-stable GPI biosynthetic intermediates and their fluorescence labeled counterparts are currently underway. This strategy provides the method of synthesis of non-hydrolysable GPI probes to study the chemical basis of the nanodomain organization of GPI-anchored proteins and cholesterol in plasma membrane to decipher the chemical basis of the GPI lipid raft organization.

Experimental section

General methods

Solvents were purified according to the standard procedures, and the reagents used were of the highest purity available. All reactions were performed in flame-dried glass apparatus under an argon/nitrogen atmosphere unless mentioned otherwise. Anhydrous solvents like CH_2Cl_2, Et_2O, THF, CH_3OH, CH_3CN, DMF, pyridine, Et_3N were freshly dried using standard methods. NMR measurements (^1H, ^{13}C and ^{31}P) were recorded on a 400 or 500 MHz NMR-spectrometer fitted with a pulse-field gradient probe, and tetramethylsilane (TMS) or residual resonance of deuterated solvent was used as the internal reference. Chemical shifts are expressed in (δ) parts per million and coupling constants J in hertz. Mass spectra were recorded either with an LCMS-QTOF instrument or with a MALDI-TOF/TOF mass spectrophotometer using 2,5-dihydroxy benzoic acid/α-cyano-4-hydroxy cinnamic acid as the matrix in acetonitrile–water containing 0.01% TFA. Optical rotations were measured on a digital polarimeter. Analytical thin layer chromatography (TLC) was performed on precoated UV-active aluminum backed silica plates, visualized with a UV254 lamp and stained with 20% phosphomolybdic acid in ethanol or 20% H_2SO_4 in methanol. Silica column chromatography was carried out with silica gel (100–200 mesh) or flash silica gel (230–400 mesh). The enzyme PI-PLC was purified as reported earlier[17] in the National Centre for Biological Sciences (Tata Institute of Fundamental Research), Bangalore.

A. Procedures and spectroscopic analytical data

1,3,4,6-Tetra-*O*-acetyl-2-azido-2-deoxy-α/β-D-glucopyranoside (4).[20] *Preparation of TfN_3*: CH_2Cl_2 (250 mL) was added to a solution of NaN_3 (59.5 g, 0.92 mol) in water (150 mL) at 0 °C. The mixture was stirred vigorously and treated with trifluoromethanesulfonic anhydride (31.0 mL, 0.19 mol) over a period of 3 h at 0 °C. After the complete addition of trifluoromethanesulfonic anhydride, the reaction mixture was stirred at 0 °C for 2.5 h. The aqueous phase was extracted with CH_2Cl_2 (2 × 100 mL) and the combined organic layers were washed with saturated Na_2CO_3 and saved for use in the next step. [***Caution*:** TfN_3 is explosive when not in solution!].

$CuSO_4$ (140 mg, 0.88 mmol) and K_2CO_3 (19.2 g, 0.14 mol) were added to a solution of glucosamine hydrochloride **3** (20.0 g, 0.092 mol) in water (300 mL). Methanol (600 mL) was added to the reaction mixture followed by the addition of the TfN_3 solution. Methanol was added until the solution was homogeneous (~300 mL). The clear blue solution was allowed to stir for 24 h at room temperature. Glycine (70 g) was added and the reaction mixture was again allowed to stir for 24 h. The glycine was filtered off and the solvent was removed *in vacuo* to afford brown oil. The oil was taken up in pyridine (95 mL), cooled to 0 °C and DMAP (~30 mg) and acetic anhydride (86 mL, 0.91 mol) were added. The solution was stirred for 12 h at room temperature. The reaction was quenched with saturated $NaHCO_3$ and the aqueous phase was extracted with CH_2Cl_2 (3 × 1000 mL). The organic phases were combined, dried over $MgSO_4$, filtered and the solvent was removed *in vacuo* to yield brown oil. Flash chromatography of hexanes–EtOAc (7 : 3) afforded a colorless oil (mixture of α/β) (27.5 g, 0.074 mol, 80%). R_f = 0.25 (hexane–EtOAc, 7 : 3); ^1H NMR (400 MHz, $CDCl_3$): δ 5.54 (d, J = 8.6 Hz, 1H), 5.10–5.00 (m, 2H), 4.29 (dd, J = 12.5, 4.5 Hz, 1H), 4.07 (dd, J = 12.5, 2.1 Hz, 1H), 3.81–3.77 (m, 1H), 3.65 (t, J = 9.5 Hz, 1H), 2.18 (s, 3H), 2.08 (s, 3H), 2.06 (s, 3H), 2.01 (s, 3H); HRMS (MALDI): calcd for $C_{14}H_{19}N_3O_9$ $(M + Na)^+$ 396.1014, found 396.1020.

3,4,6-Tri-*O*-acetyl-2-azido-2-deoxy-α/β-D-glucopyranoside (5).[20] To an ice cold solution of 1,3,4,6-tetra-*O*-acetyl-2-azido-2-deoxy-α/β-D-glucopyranoside **4** (10 g, 0.027 mol) in anhydrous DMF (100 mL) was added hydrazine acetate (4 g, 0.054 mol). After addition, the reaction mixture was warmed to room temperature and stirred for 30 min. Then it was diluted with EtOAc (200 mL) and washed with $NaHCO_3$ (2 × 100 mL) and brine solution (2 × 100 mL) respectively. The organic phase was dried over Na_2SO_4 and concentrated *in vacuo*. The crude was purified by flash chromatography eluting with hexanes–EtOAc (7 : 3) to give 7.6 g (85%) of **5** as a colorless oil. R_f = 0.20 (hexane–EtOAc, 7 : 3); HRMS (MALDI): calcd for $C_{12}H_{17}N_3O_8$ $(M + Na)^+$ 354.0908, found 354.0926.

3,4,6-Tri-*O*-acetyl-2-azido-2-deoxy-1-trichloroacetimido-α/β-D-glucopyranoside (6).[14b] To a solution of 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α/β-D-glucopyranoside **5** (2 g, 6.04 mmol) in anhydrous CH_2Cl_2 (20 ml), trichloroacetonitrile (0.72 ml, 7.2 mmol) and previously dried K_2CO_3 (1.67 g, 12.08 mmol) were added and stirred for 1 h at room temperature. The reaction mixture was filtered through a celite pad, concentrated *in vacuo* and subjected to flash chromatography with hexanes–EtOAc (7 : 3) to provide compound **6** {(α : β: 0.25 : 0.75), 2.41 g, 84% yield} as a colorless solid. R_f = 0.25 (hexane–EtOAc, 7 : 3); ^1H NMR (500 MHz, $CDCl_3$, peaks labelled for β isomer only): δ 8.82 (s, 1H), 5.72 (d, J = 8.5 Hz, 1H), 5.14–5.11 (m, 2H), 4.32 (dd, J = 12.3, 4.3 Hz, 1H), 4.13 (dd, J = 12.8, 1.9 Hz, 1H), 3.84–3.81 (m, 2H), 2.11 (s, 3H), 2.08 (s, 3H), 2.04 (s, 3H); HRMS (MALDI): calcd for $C_{14}H_{17}Cl_3N_4O_8$ $(M + Na)^+$ 497.0004, found 497.0026.

(*S*)-(−)-Dimethyl malate (11).[16] To a solution of (*S*)-(−)-malic acid **10** (7 g, 0.05 mol) in MeOH (100 mL) was added a catalytic amount of *p*-TsOH·H_2O (0.5 g) and the reaction mixture was stirred overnight at room temperature. After completion, the reaction mixture was neutralized with triethyl amine and concentrated *in vacuo*. The crude product was purified by flash chromatography eluting with CH_3COCH_3–CH_2Cl_2 (1 : 19) to give 7.28 g (86%) of **11** as a colorless oil. R_f = 0.3 (hexane–EtOAc, 7 : 3); ^1H NMR (400 MHz, $CDCl_3$): δ 4.54–4.50 (m, 1H), 3.82 (s, 3H), 3.72 (s, 3H), 3.31 (d, J = 4.3 Hz, 1H), 2.84 (qd, J = 16.4, 5.3 Hz, 2H); HRMS (ESI): calcd for $C_6H_{10}O_5$ $(M + Na)^+$ 185.0420, found 185.0422.

(*S*)-1,2,4-Butanetriol (12).[16] (*S*)-(−)-Dimethyl malate **11** (7 g, 0.04 mol) dissolved in 80 ml of dry tetrahydrofuran was added dropwise to a solution of lithium borohydride (23 mL of 2 M solution, 0.04 mol) in 20 mL of dry tetrahydrofuran with the help of a cannula under a nitrogen atmosphere (place an ice bath if more heat is generated towards the end of the

addition). After complete addition, the reaction mixture was refluxed for 2 h. Addition of water to the reaction mixture gave a white precipitate, which was filtered and washed with four 30 ml portions of dry ethanol. The combined solution was evaporated to near dryness *in vacuo*. The inorganic material contained in the residual oil was removed by short column chromatography (flash silica). [The column was first packed in chloroform followed by elution with pure acetone, and then the compound was loaded by dissolving it in MeOH–CH_3COCH_3 (1 : 9) followed by elution with a gradual increase in percentage of MeOH from MeOH–CH_3COCH_3 (1 : 9) to MeOH–CH_3COCH_3 (3 : 7)]. Removal of the solvent gave 4.35 g (95%) of **12** as a slightly yellow oil, indicated to be practically pure by NMR. R_f = 0.7 (EtOAc–MeOH–CH_3COCH_3–H_2O, 7 : 1 : 1 : 1); ^{1}H NMR (400 MHz, CD_3OD): δ 3.80–3.71 (m, 3H), 3.54–3.45 (m, 2H), 1.79–1.71 (m, 1H), 1.66–1.57 (m, 1H); HRMS (ESI): calcd for $C_4H_{10}O_3$ $(M + Na)^+$ 129.0522, found 129.0526.

(*S*)-1,2-*O*-Isopropylidenebutane-1,2,4-triol (13).[16] (*S*)-1,2,4-Butanetriol **12** (4 g, 0.037 mol) was dissolved in acetone (100 ml) and a catalytic amount of *p*-toluenesulfonic acid (0.5 g) was added to it. Then the reaction mixture was refluxed for 2.5 h. After completion, the reaction mixture was neutralized with triethyl amine and acetone was evaporated to dryness. The residue was taken up in ethyl acetate and washed with aqueous solutions of sodium bicarbonate and sodium chloride respectively, and the organic layer was dried over $MgSO_4$. After removal of the solvent, flash chromatography CH_3COCH_3–CH_2Cl_2 (2 : 8) of the residue gave 4.79 g (87%) of **13** as a colorless oil. R_f = 0.3 (hexane–EtOAc, 7 : 3); ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.31–4.24 (m, 1H), 4.10 (dd, J = 8.0, 6.0 Hz, 1H), 3.80 (t, J = 5.5 Hz, 2H), 3.60 (t, J = 7.6 Hz, 1H), 2.34 (brs, 1H), 1.85–1.80 (m, 2H), 1.43 (s, 3H), 1.37 (s, 3H); HRMS (ESI): calcd for $C_7H_{14}O_3$ $(M + Na)^+$ 169.0841, found 169.0846.

(*S*)-1-Iodo-*O*-isopropylidenebutane-3,4-diol (14a).[21] To a solution of (*S*)-1,2-*O*-isopropylidenebutane-1,2,4-triol **13** (1.46 g, 10 mmol) in toluene (20 mL) were added PPh_3 (recrystallized from acetone, 3.14 g, 12 mmol), imidazole (2.04 g, 30 mmol) and iodine (3.29 g, 13 mmol). The reaction mixture was stirred at 90 °C for 2 h. After completion of the reaction (confirmed by TLC) toluene was evaporated under reduced pressure, the residue was dissolved in CH_2Cl_2, washed with saturated $Na_2S_2O_3$ (to quench the unreacted iodine), brine and dried over Na_2SO_4. The solvent was then removed *in vacuo* and the residue was purified by flash column chromatography (hexane–ethyl acetate = 49 : 1) to afford 2.71 g (98%) of compound **14a** as a colorless oil. R_f = 0.7 (hexane–EtOAc, 9 : 1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.16–4.08 (m, 1H), 4.02 (dd, J = 7.9, 6.1 Hz, 1H), 3.52 (dd, J = 8.0, 6.5 Hz, 1H), 3.21–3.14 (m, 2H), 2.08–1.93 (m, 2H), 1.34 (s, 3H), 1.29 (s, 3H); HRMS (ESI): calcd for $C_7H_{13}IO_2$ $(M + H)^+$ 257.0033, found 257.0036.

In an identical procedure **14b**[22] was prepared from **7** (yield, 98%). R_f = 0.7 (hexane–EtOAc, 9 : 1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.31–4.25 (m, 1H), 4.15 (dd, J = 8.6, 6.1 Hz, 1H), 3.79 (dd, J = 8.6, 5.4 Hz, 1H), 3.25 (dd, J = 9.8, 4.6 Hz, 1H), 3.15 (dd, J = 9.8, 8.5 Hz, 1H), 1.46 (s, 3H), 1.35 (s, 3H); HRMS (ESI): calcd for $C_6H_{11}IO_2$ $(M + H)^+$ 242.9876, found 242.9887.

(*S*)-Diethyl-[*O*-3,4-isopropylidene-3,4-dihydroxybutyl]phosphonate (15a).[16] A mixture of **14a** (1 g, 4.78 mmol) and triethyl phosphite (4.1 mL, 23.91 mmol) was stirred at 135–150 °C for 8 h, at which time the excess triethyl phosphite was removed *in vacuo*. The residual oil was a mixture of **15a** and diethyl ethylphosphonate. The *S*-(−)-diethyl phosphonate **15a** has the same polarity as diethyl ethylphosphate and made chromatographic separation very cumbersome; therefore the oil was subjected to the next step without purification.

An identical procedure was followed for the preparation of **15b**[23] from **14b**.

(*S*)-Diethyl-(3,4-dihydroxybutyl)phosphonate (16a).[16] A solution of the above oil containing **15a** (2.14 g) and *p*-TsOH·H_2O (120 mg) in CH_3OH (30 mL) was stirred overnight at room temperature. $NaHCO_3$ was added to neutralize the solution, and the stirring was continued for another 10 min. The mixture was filtered, and the filtrate was concentrated *in vacuo*. The residue was dissolved in $CHCl_3$ (10 mL) and filtered through celite. Concentration of the filtrate left a colorless oil, which was purified by flash chromatography eluting with $CHCl_3$–CH_3OH (5 : l) to give 1 g (82% over two steps) of **16a** as a colorless oil. R_f = 0.3 ($CHCl_3$–CH_3OH, 9 : 1); ^{1}H NMR (400 MHz, CD_3OD): δ 4.15–4.06 (m, 4H), 3.63–3.57 (m, 1H), 3.46 (dd, J = 5.6, 1.9 Hz, 2H), 2.08–1.75 (m, 4H), 1.33 (t, J = 7.1 Hz, 6H); ^{31}P NMR (161 MHz, CD_3OD): δ 33.79; HRMS (ESI): calcd for $C_8H_{19}O_5P$ $(M + H)^+$ 227.1043, found 227.1046.

An identical procedure followed for the preparation of **16b**[23] from **15b** (yield, 82%). R_f = 0.3 ($CHCl_3$–CH_3OH, 9 : 1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.15–4.10 (m, 5H), 3.67 (dd, J = 11.2, 3.2 Hz, 1H), 3.53 (dd, J = 11.3, 5.8 Hz, 1H), 2.10–1.92 (m, 2H), 1.33 (t, J = 7.1 Hz, 6H); ^{31}P NMR (161 MHz, $CDCl_3$): δ 30.05; HRMS (ESI): calcd for $C_7H_{17}O_5P$ $(M + H)^+$ 213.0886, found 213.0873.

(*S*)-Diethyl 3,4-bis(palmitoyloxy)butylphosphonate (17a).[24] To a solution of **16a** (0.5 g, 2 mmol), palmitic acid (1.12 g, 4.38 mmol), and DMAP (56 mg, 0.46 mmol) in anhydrous CH_2Cl_2 (5 mL) was added DCC (0.94 g, 4.56 mmol), and the resulting mixture was stirred overnight at room temperature. After completion of the reaction a white solid was removed by filtration through Celite, and the filtrate was evaporated to furnish a white solid, which was purified by flash chromatography on silica gel using hexanes–EtOAc (1 : l) to afford 1.23 g (88%) of **17a** as a white solid. R_f = 0.6 ($CHCl_3$–CH_3OH, 9 : 1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.12–5.06 (m, 1H), 4.24 (dd, J = 11.8, 3.7 Hz, 1H), 4.14–4.01 (m, 5H), 2.32–2.28 (m, 4H), 1.98–1.68 (m, 4H), 1.65–1.55 (m, 4H), 1.34–1.26 (m, 54H), 0.88 (t, J = 7.1 Hz, 6H); ^{31}P NMR (161 MHz, $CDCl_3$): δ 30.65; ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.3, 173.1, 70.8 (d, J_{CP} = 18.1 Hz), 64.2, 61.6 (d, J_{CP} = 6.5 Hz), 34.2, 34.0, 33.9, 31.8, 29.6, 29.6, 29.5, 29.4, 29.4, 29.3, 29.2, 29.2, 29.1, 25.6, 24.9, 24.9, 24.8, 24.1 (d, J_{CP} = 4.3 Hz), 22.6, 21.6 (d, J_{CP} = 143.8 Hz), 16.4 (d, J_{CP} = 6.0 Hz), 14.03; HRMS (ESI): calcd for $C_{40}H_{79}O_7P$ $(M + H)^+$ 703.5636, found 703.5602.

An identical procedure was followed for the preparation of **17b**[25] from **16b** (yield, 88%). R_f = 0.6 ($CHCl_3$–CH_3OH, 9 : 1); ^1H NMR (400 MHz, $CDCl_3$): δ 5.39–5.30 (m, 1H), 4.37 (dd, J = 11.9, 3.2 Hz, 1H), 4.17–4.07 (m, 5H), 2.33–2.28 (m, 4H), 2.13 (dd, J = 19.0, 6.8 Hz, 1H), 1.61 (t, J = 7.0 Hz, 4H), 1.33–1.26 (m, 54H), 0.88 (t, J = 7.1 Hz, 6H); ^{31}P NMR (161 MHz, $CDCl_3$): δ 25.45; ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.2, 172.7, 66.7, 64.6 (d, J_{CP} = 8.8 Hz), 61.0 (t, J_{CP} = 6.1 Hz), 34.3, 34.1, 31.9, 29.7, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 29.1, 28.0, 27.9 (d, J_{CP} = 141.8 Hz), 24.8 (d, J_{CP} = 8.8 Hz), 22.7, 16.4 (d, J_{CP} = 6.0 Hz), 14.1; HRMS (ESI): calcd for $C_{39}H_{77}O_7P$ $(M + Na)^+$ 711.5305, found 711.5332.

(*S*)-[3,4-Bis(palmitoyloxy)butyl]phosphonic acid (18a).[26] To a solution of **17a** (0.1 g, 0.14 mmol) in anhydrous CH_2Cl_2 (1 mL) at room temperature was added slowly over 45 min bromotrimethylsilane (~150 μL). After the resulting mixture was stirred at room temperature for 6 h, the solvent was evaporated under reduced pressure. The residue was dissolved in 10% aqueous THF (1 mL), and the solution was heated at reflux for 1 h. The solvent was removed under reduced pressure, and the residue was dissolved in $CHC1_3$ (5 mL). The $CHC1_3$ solution was then dried (Na_2SO_4), filtered, and concentrated under reduced pressure to afford 92 mg (98%) of the phosphonic acid **18a** as a white solid that was utilized in the next step without further purification. R_f = 0.1 ($CHCl_3$–CH_3OH, 9 : 1); ^1H NMR (400 MHz, $CDCl_3$): δ 5.14 (brs, 1H), 4.26 (d, J = 9.2 Hz, 1H), 4.04 (brs, 1H), 2.36–2.28 (m, 4H), 1.93–1.71 (m, 4H), 1.61–1.59 (m, 4H), 1.34–1.26 (m, 48H), 0.88 (t, J = 7.1 Hz, 6H); ^{31}P NMR (161 MHz, $CDCl_3$): δ 33.66; HRMS (MALDI): calcd for $C_{36}H_{71}O_7P$ $(M + Na)^+$ 669.4830, found 669.4847.

An identical procedure was followed for the preparation of **18b**[27] from **17b** (yield, 98%). R_f = 0.1 ($CHCl_3$–CH_3OH, 9 : 1); ^1H NMR (400 MHz, $CDCl_3$): δ 5.43–5.41 (m, 1H), 4.34–4.31 (m, 1H), 4.12–4.09 (m, 1H), 2.34–2.29 (m, 4H), 2.15–2.09 (m, 2H), 1.58–1.57 (m, 4H), 1.34–1.26 (m, 48H), 0.85 (t, J = 7.1 Hz, 6H); ^{31}P NMR (161 MHz, $CDCl_3$): δ 28.91; HRMS (ESI): calcd for $C_{35}H_{69}O_7P$ $(M - H)^-$ 631.4708, found 631.4707.

(3,4,6-Tri-*O*-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl)-(1–6)-1-*O*-4-methoxybenzyl-2,3,4,5-tetra-*O*-benzyl-D-*myo*-inositol (19).[28] A mixture of 1-*O*-4-methoxybenzyl-2,3,4,5-tetra-*O*-benzyl-D-*myo*-inositol acceptor **2** (1.74 g, 2.63 mmol) and 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α/β-D glucopyranosyltrichloroacetimidate donor **6** (2.1 g, 4.41 mmol), and freshly activated powdered 4 Å molecular sieves were dried by azeotropic removal of residual moisture through toluene. The mixture was dissolved in anhydrous CH_2Cl_2 (14 mL), stirred under nitrogen at room temperature for 30 min, and then cooled to −78 °C. To the above was added a solution of TMSOTf (0.6 mL, 0.2 M in CH_2Cl_2) dropwise and the mixture was stirred further for 40 min at −78 °C. After completion, the reaction mixture was neutralized with triethylamine, filtered through celite and concentrated. The silica column chromatography (hexane–ethyl acetate) provided product **19** (2.3 g, 90%) as a colorless solid. R_f = 0.4 (hexane–EtOAc, 7 : 3); $[\alpha]_D$ = +74 (c 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.40–7.21 (m, 22H), 6.86 (dd, J = 8.7, 2.4 Hz, 2H), 5.79 (d, J = 3.6 Hz, 1H), 5.45–5.40 (m, 1H), 5.14–5.11 (m, 1H), 4.94–4.89 (m, 3H), 4.80–4.69 (m, 3H), 4.66–4.63 (m, 2H), 4.48–4.43 (m, 2H), 4.28–4.23 (m, 2H), 4.15–4.12 (m, 1H), 4.06 (s, 1H), 3.81 (s, 3H), 3.62–3.59 (m, 2H), 3.49 (dd, J = 9.5, 1.8 Hz, 1H), 3.45 (dd, J = 9.6, 1.8 Hz, 1H), 3.43–3.39 (m, 1H), 3.15–3.11 (m, 1H), 2.07 (s, 3H), 1.96 (s, 3H), 1.83 (s, 3H); HRMS (MALDI): calcd for $C_{54}H_{59}N_3O_{14}$ $(M + Na)^+$ 996.3895, found 996.3868.

(2-Azido-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1–6)-1-*O*-4-methoxybenzyl-2,3,4,5-tetra-*O*-benzyl-D-*myo*-inositol (20).[29] The preceding pseudo disaccharide **19** (1.36 g, 1.4 mmol) dissolved in a solvent mixture of anhydrous CH_2Cl_2 (3 mL) and MeOH (12 mL) was treated with a catalytic amount of NaOMe. The reaction was stirred for 24 h at room temperature. After completion, the reaction was neutralized with a cation-exchange resin, filtered and concentrated. The residue was dissolved in anhydrous DMF (32 mL) and cooled to 0 °C. This was followed by addition of benzyl bromide (2 mL) and NaH (0.6 g). The reaction was stirred at room temperature for 12 h, after which the reaction was quenched by addition of MeOH (1 mL). The reaction was diluted with ethyl acetate (100 mL), and the organic layer was washed with brine and water, dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by silica gel column chromatography (hexane–ethyl acetate) to give (1.25 g, 80%) **20**. R_f = 0.5 (hexane–EtOAc, 7 : 3); $[\alpha]_D$ = +41 (c 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.44–7.07 (m, 37H), 6.90 (d, J = 8.3 Hz, 2H), 5.79 (d, J = 3.5 Hz, 1H), 5.06–4.99 (m, 2H), 4.92–4.81 (m, 5H), 4.76–4.66 (m, 4H), 4.57–4.52 (m, 3H), 4.41 (d, J = 11.0 Hz, 1H), 4.32–4.24 (m, 2H), 4.17 (t, J = 9.6 Hz, 1H), 4.07–3.97 (m, 3H), 3.85 (s, 3H), 3.77–3.69 (m, 1H), 3.53–3.43 (m, 3H), 3.31–3.14 (m, 3H); HRMS (MALDI): calcd for $C_{69}H_{71}N_3O_{11}$ $(M + Na)^+$ 1140.4986, found 1140.4988.

(2-Azido-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1–6)-2,3,4,5-tetra-*O*-benzyl-D-*myo*-inositol (21).[14b] Compound **20** (0.25 g, 0.22 mmol) was dissolved in CH_2Cl_2–H_2O, 99 : 1 (40 mL), cooled to 0 °C, treated with DDQ (0.20 g, 0.88 mmol), and stirred at room temperature for 3 h. The reaction mixture was quenched with saturated $NaHCO_3$ solution, extracted with CH_2Cl_2 (3 × 100 mL) and the organic layer was dried over sodium sulfate. The solvent was removed under reduced pressure and the residue was purified by column chromatography (hexane–EtOAc) to afford **21** (0.12 g, 55%). R_f = 0.4 (hexane–EtOAc, 7 : 3); $[\alpha]_D$ = +45 (c 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.32–7.00 (m, 35H), 5.37 (d, J = 3.5 Hz, 1H), 4.96–4.31 (m, 14H), 4.08–4.02 (m, 2H), 3.99–3.85 (m, 3H), 3.65 (t, J = 9.6 Hz, 1H), 3.60–3.48 (m, 2H), 3.43–3.40 (m, 1H), 3.30 (t, J = 9.6 Hz, 1H), 3.15 (dd, J = 11.0, 1.8 Hz, 1H), 2.98 (d, J = 11.0, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 138.8, 138.6, 138.6, 138.2, 137.9, 137.9, 137.8, 128.4, 128.4, 128.4, 128.3, 128.3, 128.0, 127.9, 127.8, 127.7, 127.7, 127.7, 127.6, 127.6, 127.5, 127.3, 127.3, 98.4, 83.2, 81.7, 81.0, 80.6, 80.5, 78.7, 78.0, 75.7, 75.4, 75.3, 74.9, 74.8, 73.4, 73.1, 71.2, 70.6, 68.5, 63.7; HRMS (MALDI): calcd for $C_{61}H_{63}N_3O_{10}$ $(M + Na)^+$ 1020.4411, found 1020.4436.

Triethylammonium (3,4,6-tri-*O*-benzyl-2-azido-2-deoxy-α-D-glucopyranosyl)-(1–6)-2,3,4,5-tetra-*O*-benzyl-1-*O*-(*sn*-3,4-di-*O*-palmitoyl-butyl-1-phosphonate)-D-*myo*-inositol (22a). To a

suspension of the phosphonic acid **18a** (20 mg, 0.03 mmol) obtained above and glycosyl acceptor **21** (37 mg, 0.037 mmol) in dry pyridine (1 mL) at 60 °C was added trichloroacetonitrile (500 μL, 5 mmol), and the reaction mixture was stirred at 60 °C for 60 h. After completion, the reaction mixture was concentrated under reduced pressure to afford a pale orange residue. Purification of the residue by chromatography on silica gel eluting with $CHC1_3$–CH_3OH (9 : l) gave 40 mg (77%) of **22a** as a white foam. R_f = 0.6 (CH_2Cl_2–MeOH, 9 : 1); $[\alpha]_D$ = +25.33 (*c* 0.01, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34–7.20 (m, 35H), 5.67 (d, *J* = 3.4 Hz, 1H), 5.08–4.98 (m, 3H), 4.93–4.81 (m, 4H), 4.78–4.73 (m, 3H), 4.65–4.57 (m, 4H), 4.54–4.51 (m, 1H), 4.48–4.44 (m, 1H), 4.39–4.32 (m, 1H), 4.13–4.01 (m, 4H), 3.85–3.75 (m, 3H), 3.65–3.49 (m, 4H), 3.31–3.26 (m, 1H), 2.89–2.87 (m, 6H), 2.21–2.14 (m, 4H), 1.85–1.74 (m, 4H), 1.56–1.46 (m, 4H), 1.31–1.20 (m, 48H), 1.18 (t, *J* = 7.2 Hz, 9H), 0.88 (t, *J* = 7.0 Hz, 6H); ^{31}P NMR (161 MHz, $CDCl_3$): δ 23.48; ^{13}C NMR (100 MHz, $CDCl_3$): 173.3, 173.2, 139.5, 138.6, 138.6, 138.5, 138.4, 138.2, 138.1, 128.4, 128.3, 128.3, 128.2, 128.1, 128.0, 128.0, 127.9, 127.8, 127.8, 127.6, 127.5, 127.4, 127.4, 127.0, 126.9, 97.2, 84.2, 82.2, 80.8, 80.8, 80.4 (d, J_{CP} = 6.1 Hz), 78.2 (d, J_{CP} = 5.7 Hz), 77.6 (d, J_{CP} = 6.2 Hz), 75.7, 75.2, 75.1, 74.9, 73.6, 73.5, 72.3, 71.8 (d, J_{CP} = 14.8 Hz), 71.5, 70.1, 68.3, 64.4, 63.7, 45.3, 34.3, 34.3, 34.1, 31.9, 29.7, 29.7, 29.6, 29.5, 29.5, 29.4, 29.3, 29.3, 29.1, 25.0 (d, *J* = 4.9 Hz), 24.8, 22.7, 14.1, 8.5; HRMS (MALDI): calcd for $C_{97}H_{132}N_3O_{16}P$ $(M - H)^-$ 1625.9306, found 1625.9333.

Triethylammonium (3,4,6-tri-*O*-benzyl-2-azido-2-deoxy-α-D-glucopyranosyl)-(1–6)-2,3,4,5-tetra-*O*-benzyl-1-*O*-(*sn*-2,3-di-*O*-palmitoyl-propyl-1-phosphonate)-D-*myo*-inositol (22b). To a suspension of the phosphonic acid **18b** (20 mg, 0.03 mmol) obtained above and glycosyl acceptor **21** (37 mg, 0.037 mmol) in dry pyridine (1 mL) at 60 °C was added trichloroacetonitrile (500 μL, 5 mmol), and the reaction mixture was stirred at 60 °C for 60 h. After completion, the reaction mixture was concentrated under reduced pressure to afford a pale orange residue. Purification of the residue by chromatography on silica gel eluting with $CHC1_3$–CH_3OH (9 : l) gave 25 mg (50%) of **22b** as a white foam. R_f = 0.6 (CH_2Cl_2–MeOH, 9 : 1); $[\alpha]_D$ = +28.38 (*c* 0.01, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.40–7.18 (m, 35H), 5.67 (d, *J* = 3.4 Hz, 1H), 5.06–4.97 (m, 3H), 4.92 (d, *J* = 3.4 Hz, 1H), 4.85–4.75 (m, 6H), 4.65–4.56 (m, 4H), 4.48–4.44 (m, 2H), 4.39–4.34 (m, 1H), 4.14–4.00 (m, 5H), 3.83–3.74 (m, 3H), 3.65–3.53 (m, 3H), 3.35–3.31 (m, 1H), 2.98 (q, *J* = 7.0 Hz, 6H), 2.20–2.14 (m, 4H), 1.81–1.75 (m, 2H), 1.53–1.51 (m, 4H), 1.25–1.21 (m, 57H), 0.88 (t, *J* = 7.0 Hz, 6H); ^{31}P NMR (161 MHz, $CDCl_3$): δ 16.62; ^{13}C NMR (125 MHz, $CDCl_3$): 173.6, 173.2, 138.8, 138.8, 138.6, 138.5, 138.3, 138.2, 138.1, 128.5, 128.4, 128.4, 128.4, 128.3, 128.3, 128.3, 128.2, 128.2, 128.1, 128.1, 128.0, 128.0, 128.0, 127.9, 127.9, 127.8, 127.7, 127.7, 127.6, 127.6, 127.5, 127.5, 127.4, 127.3, 127.2, 127.0, 98.4, 83.0, 82.0, 81.5, 80.9, 80.5, 78.5, 77.5, 75.5, 75.4, 75.1, 74.9, 74.7, 73.6, 73.3, 72.2, 70.8, 70.1, 68.1, 64.1, 63.32, 45.3, 34.5, 34.4, 34.2, 31.9, 29.7, 29.7, 29.6, 29.5, 29.3, 29.2, 29.2, 22.7, 14.1, 8.4; HRMS (MALDI): calcd for $C_{96}H_{130}N_3O_{16}P$ $(M - H)^-$ 1611.9149, found 1611.9126.

Triethylammonium (2-amino-2-deoxy-α-D-glucopyranosyl)-(1–6)-1-*O*-(*sn*-3,4-di-*O*-palmitoyl-butyl-1-phosphonate)-D-*myo*-inositol (23a). The protected C-P GPI intermediate **22a** (0.02 g, 0.012 mmol) and the catalyst 20% $Pd(OH)_2$ (0.05 g) were dissolved in a solvent mixture of MeOH (2 mL), CH_2Cl_2 (2 mL) and H_2O (0.1 mL). The residual and the dissolved air from the flask were removed by repeated evacuations and the reaction mixture was stirred under a hydrogen atmosphere overnight. After completion of the reaction, the mixture was filtered through a small celite pad, and concentrated under reduced pressure. The product was purified by quick filtration through a silica column using MeOH–CH_2Cl_2 (1 : 1) to provide the compound **23a** (9.5 mg, 80%). R_f = 0.4 (EtOAc–MeOH–CH_3COCH_3–H_2O, 7 : 1 : 1 : 1); ^{1}H NMR (400 MHz, $CDCl_3$–CD_3OD–D_2O = 4 : 4 : 1): δ 5.47 (m, 1H), 5.17 (m, 2H), 4.34 (m, 2H), 4.15–4.03 (m, 3H), 3.87 (m, 2H), 3.73–3.67 (m, 3H), 3.39–3.36 (m, 2H), 3.19 (q, *J* = 7.0 Hz, 6H), 3.14 (t, *J* = 5.0 Hz, 1H), 2.34–2.31 (m, 4H), 2.00–1.84 (m, 4H), 1.63–1.62 (m, 4H), 1.39–1.27 (m, 57H), 0.89 (t, *J* = 7.0 Hz, 6H); ^{31}P NMR (161 MHz, $CDCl_3$–CD_3OD–D_2O = 4 : 4 : 1): δ 30.39; ^{13}C NMR (100 MHz, $CDCl_3$–CD_3OD–D_2O = 4 : 4 : 1): 175.4, 175.3, 95.4, 77.8, 73.5, 72.7, 72.6, 71.5, 70.5, 70.4, 64.8, 64.7, 62.6, 61.3, 61.2, 54.5, 46.6, 34.3, 29.6 (multiple peaks), 24.9, 22.7, 22.6, 21.8, 13.5, 8.5; HRMS (ESI): calcd for $C_{48}H_{92}NO_{16}P$ $(M - H)^-$ 968.6081, found 968.6093.

Triethylammonium (2-amino-2-deoxy-α-D-glucopyranosyl)-(1–6)-1-*O*-(*sn*-2,3-di-*O*-palmitoyl-propyl-1-phosphonate)-D-*myo*-inositol (23b). The protected C-P GPI intermediate **22b** (0.02 g, 0.012 mmol) and the catalyst 20% $Pd(OH)_2$ (0.05 g) were dissolved in a solvent mixture of MeOH (2 mL), CH_2Cl_2 (2 mL) and H_2O (0.1 mL). The residual and the dissolved air from the flask were removed by repeated evacuations and the reaction mixture was stirred under a hydrogen atmosphere overnight. After completion of the reaction, the mixture was filtered through a small celite pad and concentrated under reduced pressure. The product was purified by quick filtration through a silica column using MeOH–CH_2Cl_2 (1 : 1) to provide the compound **23b** (9.3 mg, 80%). R_f = 0.4 (EtOAc–MeOH–CH_3COCH_3–H_2O, 7 : 1 : 1 : 1); ^{1}H NMR (400 MHz, $CDCl_3$–CD_3OD–D_2O = 4 : 4 : 1): δ 5.44 (m, 2H), 4.47–4.35 (m, 2H), 4.12–3.68 (m, 9H), 3.46–3.35 (m, 2H), 3.19 (q, *J* = 7.0 Hz, 7H), 2.35–2.33 (m, 4H), 2.19–2.14 (m, 2H), 1.63–1.62 (m, 4H), 1.38–1.28 (m, 57H), 0.89 (t, *J* = 7.0 Hz, 6H); ^{31}P NMR (161 MHz, $CDCl_3$–CD_3OD–D_2O = 4 : 4 : 1): δ 23.53; ^{13}C NMR (100 MHz, $CDCl_3$–CD_3OD–D_2O = 4 : 4 : 1): 174.4, 174.3, 95.1, 77.7, 75.6, 72.9, 72.3, 71.8, 70.4, 70.0, 66.8, 64.9, 64.8, 60.7, 60.6, 54.1, 46.2, 33.6, 31.8–29.0, 28.5, 24.3, 22.1, 13.2, 7.9; HRMS (ESI): calcd for $C_{47}H_{90}NO_{16}P$ $(M + H)^+$ 956.6075, found 956.6042.

B. Method for the PI-PLC reaction[17–19]

The sample in $CHCl_3$ was evaporated and re-suspended in H_2O by bath sonication (5 min) such that the final concentration of the sample was 10 mM. To 5 μL of the sample (10 mM) were added 5 μL of sodium deoxycholate (0.8%, w/v), 10 μL of borate buffer [0.1 M (sodium borate/HCl (pH 7.5))] and 4 μL of enzyme PI-PLC. The reaction mixture was incubated at 37 °C for 20 minutes and quenched with 200 μL $CHCl_3$–MeOH–

Conc. HCl (66 : 33 : 1). The $CHCl_3$ layer was then checked for monitoring the progress of the reaction by TLC and mass spectrometry and the results are given in the Discussion part and Fig. 1.

C. Method for the PI-PLC reaction and inhibitor reactions[17–19]

NBD-GPI **26** is added to borate buffer pH 7.5 containing deoxycholate at 2 mM (above CMC concentration to form micelles). To this a PI-PLC (0.0013 mg mL^{-1}) enzyme is added and warmed to 37 degrees to initiate the reaction. The reaction is continued for 10 min for 50% of GPI-NBD to undergo PI-PLC cleavage. The reaction is terminated by placing the reaction mixture on ice. The reaction mixture is immediately spotted on TLC sheets (silica gel 60 coated on glass plates) and run in the 7 : 1 : 1 : 1 EtOAc–MeOH–Me_2CO–H_2O solvent system. This TLC is further imaged in UV to find the concentration of the product formed in each case. GPI-NBD on PI-PLC cleavage gives rise to 2 products namely the glycan part and the NBD-glycerolipid of which the latter will fluoresce in UV. Hence from the fluorescent bands of reactants (R) and the product (P) we calculate the percentage of the reaction ($P/R \times 100$). Both the reactant band and the product band are normalized to the loading control in each lane.

In the case of inhibition reactions, to the above GPI-NBD reaction, varying concentrations of the inhibitor (*i.e.*, 7.5 mM −0.1 mM in case of **23a** and 20 mM–0.1 mM in case of **23b**) are added and the reaction carried out at 37 degrees for 10 minutes. For each concentration of the inhibitor added, the corresponding % of reaction is calculated and this is subtracted from that of the NBD-GPI **26** reaction (control reaction, without inhibitor) to calculate the % of inhibition.

Acknowledgements

The financial support by CSIR research grant # BSC 0108 is gratefully acknowledged. MY, VS and SD thank CSIR for Research Fellowships. The authors thank the Instrumentation Division of CSIR-IIIM for support.

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