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Synthesis and Biological Evaluation of L-α-Phosphatidyl-D-3-deoxy-3-heteromethyl-*myo*-inositols as Phosphoinositide 3-Kinase Inhibitors

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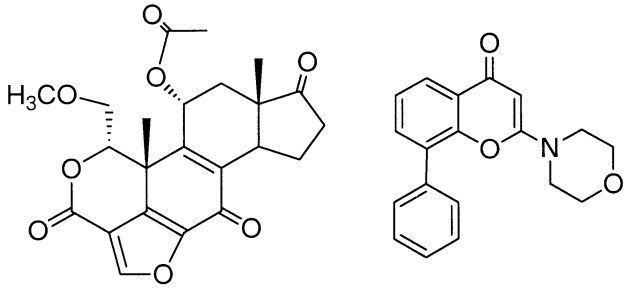
Abstract—We have synthesized a series of 3-deoxy-3-heteromethyl derivatives of L-α-phosphatidyl-D-*myo*-inositol as part of our effort to develop specific, reversible inhibitors of phosphoinositide (PI) 3-kinase. Among various derivatives examined, phosphatidyl-D-3-deoxy-3-aminomethyl-*myo*-inositol displays the highest potency in inhibiting PI 3-kinase both in vitro and in cells. It effectively suppressed antigen-stimulated degranulation in mast cells (IC_{50}, 17 μM), suggesting a potential application of this PI 3-kinase inhibitor as a mast cell-stabilizing agent.

Introduction

The pivotal role of PI 3-kinase in the regulation of diverse physiological functions has been well documented.[1–3] Cellular responses that are mediated by PI 3-kinase include cell growth and differentiation,[4,5] cell survival,[6] platelet aggregation,[7,8] T-cell activation,[6] mast cell degranulation,[9] membrane trafficking,[10] and so forth. In stimulated cells, PI 3-kinase phosphorylates the 3-OH of the inositol ring of phosphoinositides, resulting in transient accumulations of phosphatidylinositol 3,4,5-trisphosphate [PI(3,4,5)P_3] and phosphatidylinositol 3,4-bisphosphate [PI(3,4)P_2]. These lipid second messengers act as both membrane anchors and allosteric regulators, serving to recruit and activate downstream enzymes and their protein substrates. Through this membrane recruitment process, PI 3-kinase behaves as a molecular switch to regulate downstream signaling pathways that culminate in various physiological responses. Concerning potential therapeutic applications, PI 3-kinase can be targeted to block undesired cell proliferation or activation under pathological conditions.

In the literature, several types of PI 3-kinase inhibitors have been reported,[11] among which wortmannin and LY294002 have received wide attention. These two PI 3-kinase inhibitors are structurally and mechanistically distinct molecules (Fig. 1).

Wortmannin, a fungal metabolite, is a highly potent inhibitor (IC_{50}, 2 nM) that covalently modifies the Lys-802 of the p110 catalytic subunit, thereby preventing the binding of PI(4,5)P_2 and ATP to the enzyme.[12] On the other hand, LY294002 [2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one] inhibits PI 3-kinase (IC_{50}, 1.4 μM) by blocking the ATP-binding site of the enzyme.[13] More recently, Kozikowski and coworkers developed various 3-substituted derivatives of phosphatidylinositol that acted as anti-proliferative agents against cancer cell growth.[11,14,15] In our laboratory, we have embarked on



Wortmannin **LY294002**

Figure 1. Structures of wortmannin and LY294002.

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the development of PI 3-kinase inhibitors as anti-allergic agents in light of the involvement of this enzyme in mast cell activation.[16] Our data suggest that PI 3-kinase plays a key role in mast cell degranulation by stimulating Ca^{2+} influx via a phosphatidylinositol 3,4,5-trisphosphate [PI(3,4,5)P_3]-sensitive Ca^{2+} entry mechanism.[16] Here, we report the synthesis of a series of the 3-deoxy-3-heteromethyl analogues of L-α-phosphatidyl-D-*myo*-inositol (**1**–**6**) (Fig. 2) and their activity against PI 3-kinase both in vitro and in cells.

Among these analogues, L-α-phosphatidyl-D-3-deoxy-3-aminomethyl-*myo*-inositol (**5**) displayed the highest potency in PI 3-kinase inhibition, and effectively inhibited antigen-stimulated degranulation in mast cells, a process leading to allergic and inflammatory responses.

Results and Discussion

Synthesis

The aforementioned D-3-deoxy-3-heteromethyl-*myo*-inositol derivatives (**1**–**6**) were synthesized using (+)-**7** as a precursor (Fig. 3), which was used as an intermediate in our previous synthesis of PI(3)P.[17]

The efficient use of (+)-**7** was made of by converting it to the ketone (−)-**8** via DMSO-Ac_2O oxidation as described,[18] followed by the Wittig reaction to afford the olefin (−)-**9** by reacting with triphenylphosphonium methylide in the presence of **1** molar equivalent of *t*-butoxide. ^{1}H NMR analysis of the protons at C-2 and C-4 of both **8** and **9** showed no appreciable epimerization during the alkene formation. Hydroboration of **9** with 9-BBN (9-borabicyclo[3,3,1]nonane) followed by oxidation with alkaline H_2O_2 solution gave the sterically more favorable isomer (−)-**10** as a single product after chromatography. The C-3 stereochemistry of **10** was confirmed by comparing its ^{1}H NMR signal (2.63–2.68, m, 1H) with that reported for the C-3 protons in both *R* and *S* configurations.[15] Compound **10** served as a versatile intermediate for the synthesis of various 3-heteromethyl derivatives. The 3-fluoromethyl counterpart (+)-**11** was obtained by reacting **10** with DAST (diethylaminosulfur trifluoride), while (+)-**12** to (−)-**15** were yielded by treating **10** with triphenylphosphine in the presence of CCl_4, CBr_4, I_2, and $Zn(N_3)_2$·Py_2/DEAD (diethylazodicarboxylate), respectively. In addition, **10** underwent *O*-benzylation to generate (−)-**16**. These 3-heteromethyl analogues (**11**–**16**) were exposed to trifluoroacetic acid (TFA) in CH_2Cl_2 to remove the *p*-methoxybenzyl group to give the key compounds (−)-**17** to (+)-**22**. Reaction of these intermediates with 1,2-dipalmitoyl-*sn*-glycerol 3-(benzyl *N*,*N*-diisopropylphosphoramidite) (**23**) in the presence of 1*H*-tetrazole, followed by *m*-chloroperoxybenzoic acid (*m*-CPBA), gave the perbenzylated derivatives (+)-**24** to (+)-**29** that underwent hydrogenolysis to generate the target molecules **1**–**6**, with overall yields from (+)-**7** ranging from 25 to 35%.

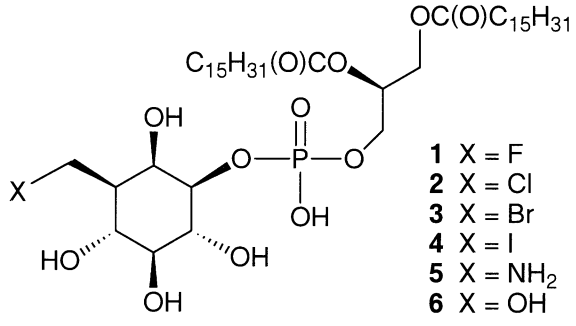

Figure 2. Structures of phosphatidyl-D-3-deoxy-3-heteromethyl-*myo*-inositols (**1**–**6**).

PI 3-kinase inhibition

The potency of the 3-deoxy-3-heteromethyl derivatives (**1**–**6**) in enzyme inhibition was examined by exposing immunoprecipitated rat liver PI 3-kinase to PI(4,5)P_2 and [γ-^{32}P]-ATP in the presence of individual compounds at different concentrations. ^{32}P-Labeled PI(3,4,5)P_3 derived from the enzymatic 3-*O*-phosphorylation of PI(4,5)P_2 was extracted and analyzed by thin layer chromatography, followed by autoradiography. Figure 4A depicts the effect of compounds **1**–**6** at 50 μM on [^{32}P]PI(3,4,5)P_3 production. Among these six inhibitors, the 3-deoxy-3-aminomethy derivative (**5**) was most potent (>98% inhibition), followed by the 3-deoxy-3-hydroxymethyl counterpart (**6**) (85% inhibition), whereas the 3-halomethyl derivatives (**1**–**4**) exhibited a modest activity in blocking [^{32}P]PI(3,4,5)P_3 production (55–65% inhibition).

According to the dose–response curves (Fig. 4B), the IC_{50} values of compounds **5** and **6** were 20±1 and 35±2 μM, respectively (n=3). It is worth noting that both **5** and **6** were not substrates for PI 3-kinase. Our preliminary kinetic data suggest that this inhibition was attributable to competitive binding with the substrate to the enzyme active site.

Effect of phosphatidyl-D-3-deoxy-3-aminomethyl-*myo*-inositol (5) on IgE-induced degranulation in mast cells. The effect of the synthetic PI 3-kinase inhibitor was tested on the degranulation of mast cells. Upon activation by antigens, mast cells immediately extrude granule-associated mediators that induce immediate allergic inflammation.[19] Published data indicate that inhibition of PI 3-kinase could effectively block the IgE-induced secretion of inflammatory mediators,[9,20] establishing a link between PI 3-kinase and mast cell activation. The inhibitory effect of **5** was assessed on antigen-induced secretion of hexosaminidase in IgE-sensitized rat basophilic leukemia (RBL-2H3) cells, a tumor analogue of rat mucosal mast cells.[21] In this study, we chose LY294002 as a control in lieu of wortmannin. Wortmannin has been shown to inhibit myosin light-chain kinase, one of the key regulatory enzymes in mast cells.[20]

After sensitizing RBL-2H3 cells with dinitrophenol (DNP)-specific monoclonal IgE, the cells were incubated with LY294002 or compound **5** at various concentrations

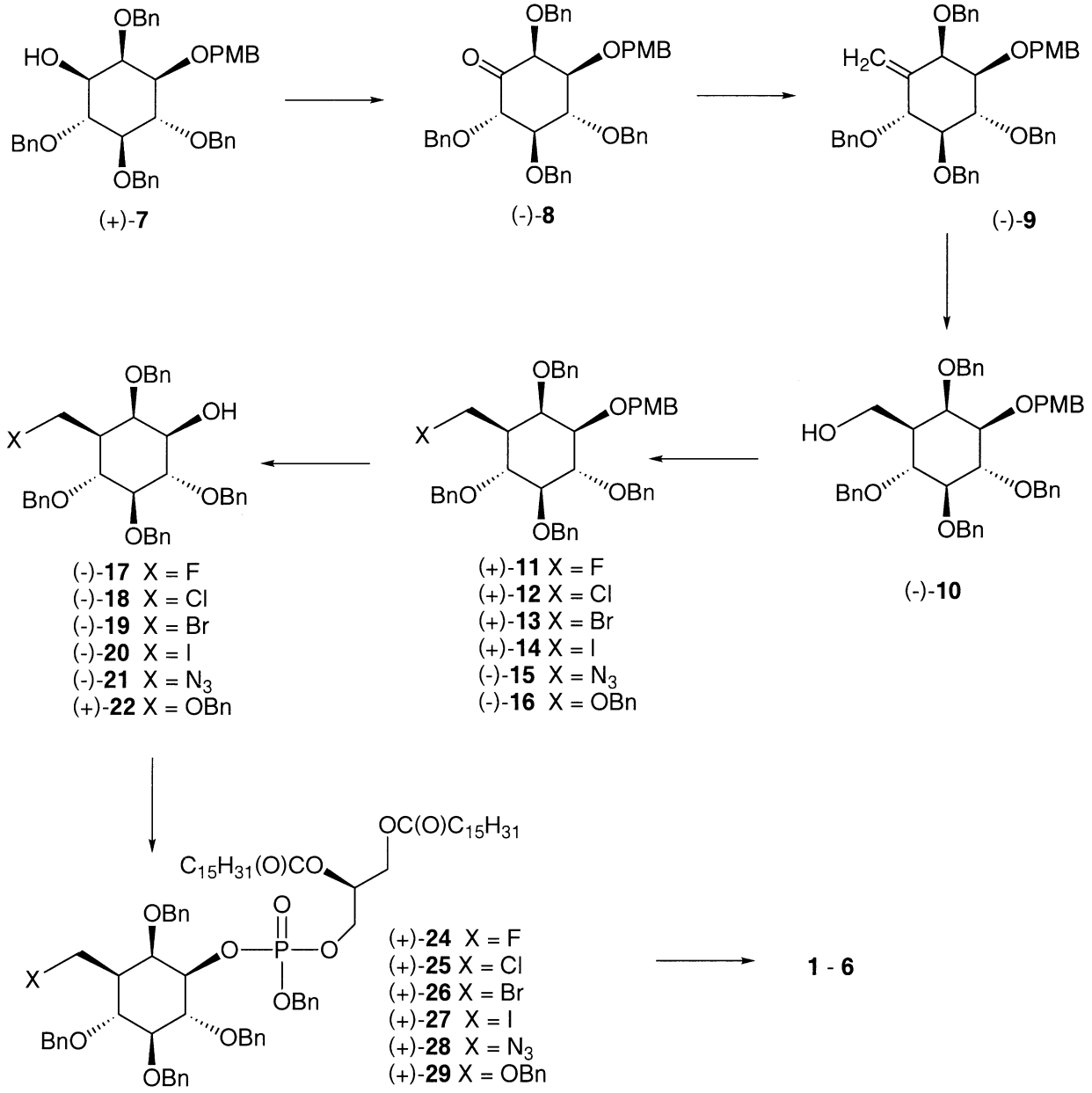

Figure 3. General scheme for the synthesis of phosphatidyl-D-3-deoxy-3-heteromethyl-*myo*-inositols (**1**–**6**).

at 37 °C for 15 min. The cells were then stimulated with the antigen DNP-human serum albumin (HAS) conjugates for 1 h, and the release of hexosaminidase into medium was determined as an index of degranulation. As shown in Figure 5, both LY294002 and **5** inhibited hexosaminidase release in a dose-dependent manner. The respective IC_{50} values were 1.8 and 17 μM, respectively, which were in line with those for PI 3-kinase inhibition in vitro, that is 1.4 and 20 μM. This observation suggests that both inhibitors could readily cross cell membranes to access intracellular PI 3-kinase.

Conclusion

The present study demonstrates the biochemical utility of compound **5** as a PI 3-kinase inhibitor and mast cell-stabilizing agent. It is noteworthy that the mechanism underlying the inhibitory effect of compound **5** on PI 3-kinase is different from that of wortmannin and LY294002. Thus, it provides an additional tool to study the physiological function of PI 3-kinase in vivo. Further studies of the effect of compound **5** on the mast cell physiology are currently under way in this laboratory.

Experimental

Material and methods

(+)-2,4,5,6-Tetra-*O*-benzyl-1-*O*-(*p*-methoxybenzyl)-*myo*-inositol (**7**) was synthesized from (+)-1,2:5,6-dicyclohexylidene-*myo*-inositol as previously described.[17] 1,2-Di-*O*-palmitoyl-*sn*-glycerol 3-(benzyl *N*,*N*-diisopropylphosphoramidite) (**23**) was prepared using a method described by Dreef et al.[22] DNP–HSA was purchased

from Sigma, and DNP-specific monoclonal IgE was a kind gift from Dr. Henry Metzger (National Institutes of Health). Monoclonal antibody against the p85 subunit of PI 3-kinase was obtained from Transduction Laboratory. [γ-^{32}P]ATP was purchased from NEN Life Science Products. PI 3-kinase was prepared by immunoprecipitation from rat liver cytosol using anti-p85 antibody according to a reported procedure.[23]

(−)-2,4,5,6-Tetra-*O*-benzyl-1-*O*-(*p*-methoxybenzyl)-3-deoxy-3-oxo-*myo*-inositol (8). A solution of (+)-**7** (1016 mg, 1.5 mmol) in dry DMSO (10 mL) was stirred with acetic anhydride (3.2 mL, 31.3 mmol) under argon at 23 °C overnight. The reaction mixture was stirred with saturated $NaHCO_3$ solution for 3 h, and extracted with CH_2Cl_2 (3×20 mL). The organic phase was washed with brine, and concentrated. Column chromatography (ether–hexane 1:10) of the residue gave **8** (amorphous, 912 mg, 90%): $[\alpha]_D^{20} = -9.6°$ (*c* 2, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 3.43 (dd, *J* = 3, 9.6 Hz, 1H), 3.49 (t, *J* = 9 Hz, 1H), 3.73–3.94 (m, 5H), 4.26 (t, *J* = 9 Hz, 1H), 4.42–4.90 (m, 10H), 6.83 (d, *J* = 8.4 Hz, 2 H), 7.18–7.38 (m, 22H); HR-MALDI (positive) calcd *m/z* for $C_{42}H_{42}O_7$: 658.2931. Found 681.2828 ($M + Na^+$).

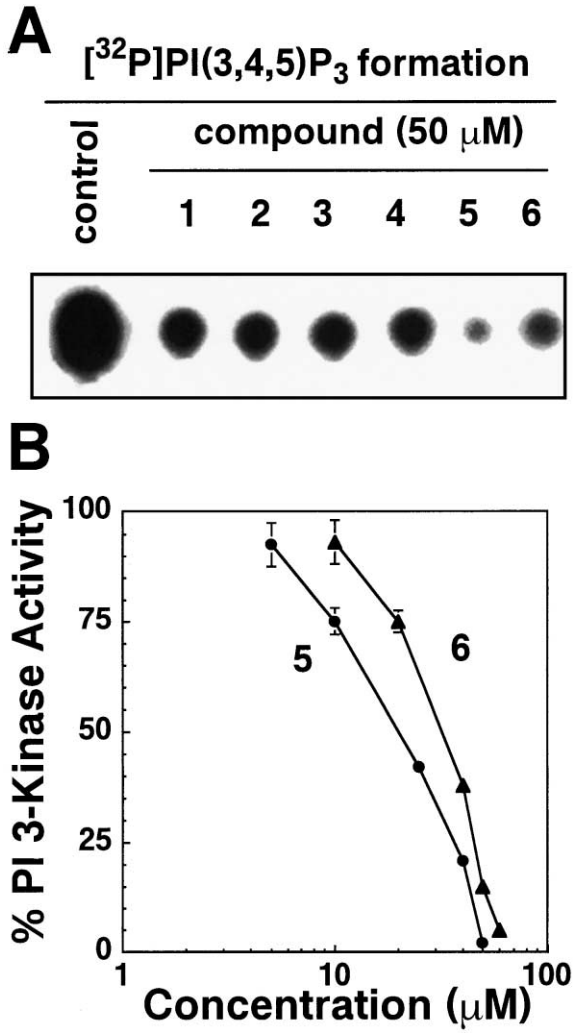

Figure 4. (A) Effect of various phosphatidyl-D-3-deoxy-3-heteromethyl-*myo*-inositols (**1**–**6**) at 50 μM on PI 3-kinase. Lane A represents the control in which only solvent carrier was added to the assay. The autoradiogram is the representative of three independent experiments. (B) Dose-dependent effect of compounds **5** and **6** on PI 3-kinase. Each data point represents the means + SD (*n* = 3).

(−)-2,4,5,6-Tetra-*O*-benzyl-1-*O*-(*p*-methoxybenzyl)-3-deoxy-3-methylene-*myo*-inositol (9). A mixture of potassium *t*-butoxide (135 mg, 1.2 mmol) and methyl-triphenylphosphine bromide (643 mg, 1.8 mmol) in dry toluene (4 mL) was stirred under reflux for 30 min (−)-**8** (789 mg, 1.2 mmol) was added in one pot. The reaction mixture was stirred at 100–110 °C for 2 h, cooled to room temperature, diluted with water, extracted with ethyl acetate (3×20 mL), dried, and concentrated. Column chromatography (ether–hexane 1:15) of the residue gave **9** (syrup, 624 mg, 80%): $[\alpha]_D^{20}$ −4.9° (*c* 2, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 3.35–3.42 (m, 2H), 3.80 (s, 3H), 4.04–4.12 (m, 2H), 4.23–4.30 (m, 2H), 4.50–4.54 (m, 3H), 4.65(q, *J* = 11.4, 13.5 Hz, 2H), 4.82–4.97 (m, 4H), 5.02 (t, *J* = 1.5 Hz), 5.43 (t, *J* = 1.5 Hz, 1H), 6.83 (d, *J* = 8.7 Hz, 2H), 7.24 (d, *J* = 8.7 Hz, 2H), 7.26–7.36 (m, 20H); HR-MALDI (positive) calcd *m/z* for $C_{43}H_{46}O_7$: 674.3244. Found: 697.3141, ($M + Na^+$), 713.2881 ($M + K^+$).

(−)-2,4,5,6-Tetra-*O*-benzyl-1-*O*-(*p*-methoxybenzyl)-3-deoxy-3-hydroxymethyl-*myo*-inositol (10). A solution of (−)-**9** (524 mg, 0.8 mmol) in dry benzene (2 mL) was added 9-BBN (1.69 mL, 0.5 M solution in THF) at room temperature, and stirred under argon overnight. Methanol (0.51 mL), 2 N NaOH (0.17 mL), and H_2O_2 (0.34 mL) were added in tandem. The mixture was stirred at 55 °C for 2 h, and concentrated. The residue was taken up with ether, washed with brine, dried and concentrated. Column chromatography (ether–hexane 1:10) of the residue gave (−)-**10** (syrup, 384 mg, 71%) as the single product: $[\alpha]_D^{20}$ −5.1° (*c* 3, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.91 (br s, 1H), 2.46–2.58 (m, 1H), 3.31 (dd, *J* = 4.5, 10.8 Hz, 1H), 3.41 (dd, *J* = 3.0, 9.6 Hz, 1H), 3.61 (t, *J* = 3.0 Hz, 1H), 3.72–3.84 (m, 5H), 3.95 (t, *J* = 8.7 Hz, 1H), 4.03 (d, *J* = 6.3 Hz, 1H), 4.47–4.92 (m, 10H), 6.84 (d, *J* = 8.7 Hz, 2H), 7.24 (d, *J* = 8.7 Hz, 2H), 7.25–7.33 (m, 20H); HR-MALDI (positive) calcd *m/z* for

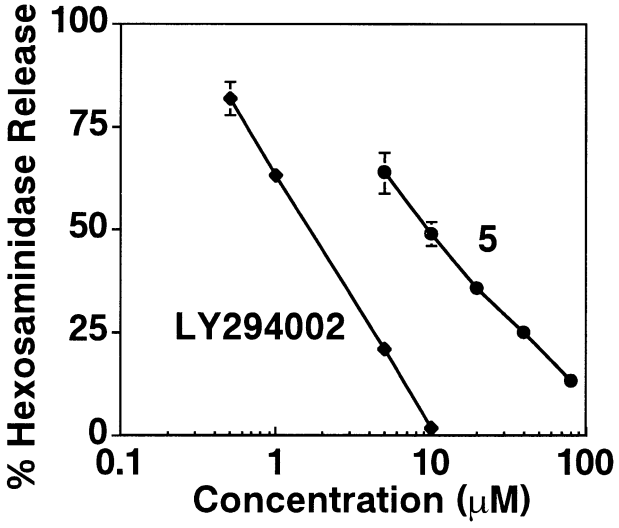

Figure 5. Dose-dependent effect of phosphatidyl-D-3-deoxy-3-aminomethyl-*myo*-inositol (**5**) and LY294002 on antigen-stimulated secretion of β-hexosaminidase from RBL-2H3 mast cells.

$C_{43}H_{44}O_6$ 656.3138. Found 679.3036, $(M+Na^+)$, 695.2775 $(M+K^+)$.

(+)-2,4,5,6-Tetra-*O*-benzyl-1-*O*-(*p*-methoxybenzyl)-3-deoxy-3-fluoromethyl-*myo*-inositol (11). A solution of (−)-**10** (37 mg, 0.055 mmol) in CH_2Cl_2 (1 mL) was added dropwise to a stirred solution of DAST (7.55 μL, 60.4 mmol) in CH_2Cl_2 (1 mL) under Ar at 0 °C within a period of 1 h. The mixture was warmed up to 25 °C, stirred for another 1 h, and poured into ice-cold water. The organic phase was washed with water, dried, and concentrated. Column chromatography (ether–hexane 1:15) of the residue gave (+)-**11** (syrup, 28 mg, 75%): $[\alpha]_D^{20}$ +8.3° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 2.63–2.68 (m, 1H), 3.38–3.50 (m, 2H), 3.68–3.73 (m, 1H), 3.79 (s, 3H), 3.83–3.92 (m, 3H), 3.99–4.04 (t, *J* = 3.3 Hz, 1H), 4.30–4.69 (m, 10H), 6.80 (d, *J* = 8.7 Hz, 2H), 7.22 (d, *J* = 8.7 Hz, 2H), 7.25–7.33 (m, 20H); HR-MALDI (positive) calcd *m*/*z* for $C_{43}H_{45}O_6F$: 676.3200. Found 699.3098 $(M+Na^+)$.

(+)-2,4,5,6-Tetra-*O*-benzyl-1-*O*-(*p*-methoxybenzyl)-3-deoxy-3-chloromethyl-*myo*-inositol (12). A solution of (+)-**10** (37 mg, 0.055 mmol) in dry pyridine (1 mL) was treated with triphenylphosphine (28.8 mg, 0.11 mmol), followed by CCl_4 (5.3 μL, 0.055 mmol) in several portions at 0 °C. The resulting mixture was heated to 70 °C, stirred for 1 h, cooled to room temperature, and diluted with ether (25 mL). The solution was washed, in tandem, with 0.5% HCl solution, saturated $NaHCO_3$ solution, and brine, dried and concentrated. Column chromatography (ether–hexane 1:15) of the residue gave (+)-**12** (syrup, 27 mg, 70%): $[\alpha]_D^{20}$ +4.7° (*c* 2, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 2.52–2.58 (m, 1H), 3.26 (t, *J* = 11.4 Hz,1H), 3.47–3.59 (m, 3H), 3.80 (s, 3H), 3.83–4.03 (m, 2H), 4.08 (t, *J* = 3.3 Hz, 1H), 4.43–4.97 (m, 10H), 6.84 (d, *J* = 8.7 Hz, 2H), 7.25 (d, *J* = 8.7 Hz, 2H), 7.29–7.35 (m, 20H); HR-MALDI (positive) calcd *m*/*z* for $C_{43}H_{45}O_6Cl$: 692.2905. Found: 715.2802 $(M+Na^+)$, 731.2542 $(M+K^+)$.

(+)-2,4,5,6-Tetra-*O*-benzyl-1-*O*-(*p*-methoxybenzyl)-3-deoxy-3-bromomethyl-*myo*-inositol (13). A solution of (+)-**10** (37 mg, 0.055 mmol) in dry pyridine (1 mL) was treated with triphenylphosphine (28.8 mg, 0.11 mmol), followed by CBr_4 (18.2 mg, 0.055 mmol) in several portions at 0 °C. The resulting mixture was heated to 70 °C for 1 h, stirred for 1 h, cooled to room temperature, and diluted with ether (25 mL). The solution was washed, in tandem, with 0.5% HCl solution, saturated $NaHCO_3$ solution, and brine, dried and concentrated. Column chromatography (ether–hexane 1:15) of the residue gave (+)-**13** (syrup, 32 mg, 79%): $[\alpha]_D^{20}$ +6.8° (*c* 3, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 2.52–2.60 (m, 1H), 3.07 (t, *J* = 11.7 Hz, 1H), 3.35–3.59 (m, 3H), 3.68–3.80 (m, 1H), 3.80 (s, 3H), 3.90–3.93 (m, 1H), 4.11 (t, *J* = 3.3 Hz, 1H), 4.45–4.97 (m, 10H), 6.85 (d, *J* = 8.7 Hz, 2H), 7.25 (d, *J* = 8.7 Hz, 2H), 7.26–7.35 (m, 2H); HR-MALDI (positive) calcd *m*/*z* for $C_{43}H_{45}O_6Br$: 736.2400. Found: 759.2297 $(M+Na^+)$, 775.2037 $(M+K^+)$.

(+)-2,4,5,6-Tetra-*O*-benzyl-1-*O*-(*p*-methoxybenzyl)-3-deoxy-3-iodomethyl-*myo*-inositol (14). A mixture of (+)-**10** (37 mg, 0.055 mmol), triphenylphosphine (18.8 mg, 0.075 mmol), and imidazole (5.2 mg, 0.075 mmol) in toluene (2 mL) was stirred at 0 °C, and I_2 (21 mg, 0.079 mmol) was slowly added. After 45 min, the solution was warmed to 70 °C and stirred for another hour, cooled to room temperature, and diluted with ether (25 mL). The solution was washed, in tandem, with 0.5% HCl solution, saturated $NaHCO_3$ solution, and brine, dried, and concentrated. Column chromatography ether–hexane 1:15) of the residue gave (+)-**14** (syrup, 30 mg, 70%): $[\alpha]_D^{20}$ +7.7° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 2.52–2.58 (m, 1H), 2.74 (t, *J* = 11.7 Hz, 1H), 3.42–3.65 (m, 4H), 3.80 (s, 3H), 3.91 (t, *J* = 9.6 Hz, 1H), 4.08 (t, *J* = 3.3 Hz, 1H), 4.46–4.95 (m, 10H), 6.83 (d, *J* = 8.7 Hz, 2H), 7.25 (d, *J* = 8.7 Hz, 2H), 7.29–7.35 (m, 20H); HR-MALDI (positive) calcd *m*/*z* for $C_{43}H_{45}O_6I$: 784.2261. Found: 807.2159 $(M+Na^+)$, 823.1898 $(M+K^+)$.

(−)-2,4,5,6-Tetra-*O*-benzyl-1-*O*-(*p*-methoxybenzyl)-3-deoxy-3-azidomethyl-*myo*-inositol (15). A mixture of (+)-**10** (24 mg, 0.036 mmol), triphenylphosphine (19 mg, 0.075 mmol) in toluene (2 mL) was stirred at 0 °C, and $Zn(N_3)_2{\cdot}Py_2$ (8.4 mg, 0.027 mmol) followed by DEAD (12.6 mg, 0.072 mol) were added. The solution was stirred at 80 °C for 4 h, cooled to room temperature, and concentrated. Column chromatography (ether–hexane 1:15) of the residue afforded (−)-**15** (syrup, 20 mg, 80%): $[\alpha]_D^{20}$ −10.2° (*c* 2, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 2.61–2.68 (m, 1H), 3.45–3.52 (m, 1H), 3.56–3.61 (m, 1H), 3.65–3.71 (m, 1H), 3.80 (s, 3H), 3.85 (t, *J* = 2.7 Hz, 1H), 3.94–4.03 (m, 1H), 4.13–4.21 (m, 2H), 4.40–5.04 (m, 10H), 6.80 (d, *J* = 8.4 Hz, 2H), 7.25–7.38 (m, 22H); HR-MALDI (positive) calcd *m*/*z* for $C_{43}H_{45}O_6N_3$: 699.3308. Found: 722.3206 $(M+Na^+)$.

(−)-2,4,5,6-Tetra-*O*-benzyl-1-*O*-(*p*-methoxybenzyl)-3-deoxy-3-benzyloxy-methyl-*myo*-inositol (16). A solution of (+)-**10** (36 mg, 0.053 mmol) in DMF (1 mL) was treated with NaH (3 mg, 85%, 0.11 mmol) at 0 °C for 30 min, followed benzyl bromide (12 μL, 0.08 mmol). The mixture was stirred 40 °C for 1 h. Excess NaH was destroyed by CH_3OH. The solution was diluted with ethyl acetate (20 mL), washed with water, dried, and concentrated. Column chromatography (ether–hexane 1:25) of the residue afforded (−)-**16** (syrup, 39 mg, 96%); $[\alpha]_D^{20}$ −13.0° (*c* 2, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 2.34–2.41 (m, 1H), 3.01–3.09 (m, 1H), 3.44–3.69 (m, 3H), 3.80 (s, 3H), 3.89–3.97 (m, 2H), 4.02 (dd, *J* = 4.2, 6 Hz, 1H), 4.43–4.90 (m, 12H), 6.85 (d, *J* = 8.4 Hz, 2H), 7.24 (d, *J* = 8.4 Hz, 2H), 7.25–7.36 (m, 25H); HR-MALDI (positive) calcd *m*/*z* for $C_{50}H_{52}O_7$: 764.3713. Found: 787.3612 $(M+Na^+)$.

(−)-2,4,5,6-Tetra-*O*-benzyl-3-deoxy-3-fluoromethyl-*myo*-inositol (17). Removal of the *p*-methoxybenzyl group of (+)-**11** (25 mg, 0.037 mmol) was achieved by exposing to CH_2Cl_2–TFA–CH_3OH (3:1.5:0.5, 5 mL) with stirring at 0 °C for 1 h. The mixture was diluted with CH_2Cl_2 (20 mL), washed, in tandem, with saturated $NaHCO_3$ solution and brine, dried and concentrated. Column chromatography (ether–hexane 1:10) of the residue gave (−)-**17** (syrup, 18.5 mg, 90%); $[\alpha]_D^{20}$ −5.3° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 2.01 (br s, 1H), 2.64–2.68 (m, 1H),

3.50–3.53 (m, 1H), 3.68–3.75 (m, 2H), 3.90 (d, $J=7.8$ Hz, 1H), 4.02 (dd, $J=5.1$, 7.8 Hz, 1H), 4.30 (d, $J=3.9$ Hz, 1H), 4.42–4.62 (m, 9H), 7.25–7.36 (m, 20H); HR-MALDI (positive) calcd m/z for $C_{35}H_{37}O_5F$: 556.2625. Found: 579.2523 ($M+Na^+$).

(−)-2,4,5,6-Tetra-*O*-benzyl-3-deoxy-3-chloromethyl-*myo*-inositol (18). Removal of the *p*-methoxybenzyl group of (+)-**12** (24 mg, 0.033 mmol) with TFA, as described for **17**, gave (−)-**18** (syrup, 18.5 mg, 92%); $[\alpha]_D^{20}$ −7.6° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 2.14 (br s, 1H), 2.56–2.62 (m, 1H), 3.47 (t, $J=10.8$ Hz, 1H), 3.54–3.62 (m, 2H), 3.75 (t, $J=8.1$ Hz, 1H), 3.86–4.04 (m, 2H), 4.06 (t, $J=4.2$ Hz, 1H), 4.45–5.01 (m, 8H), 7.26–7.35 (m, 20H); HR-MALDI (positive) calcd m/z for $C_{35}H_{37}O_5Cl$: 572.2330. Found: 595.2228, ($M+Na^+$).

(−)-2,4,5,6-Tetra-*O*-benzyl-3-deoxy-3-bromomethyl-*myo*-inositol (19). Removal of the *p*-methoxybenzyl group (+)-**13** (28 mg, 0.038 mmol) with TFA, as described for **17**, gave (−)-**19** (syrup, 21 mg, 90%); $[\alpha]_D^{20}$ −3.2° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 2.50 (br s, 1H), 2.59–2.66 (m, 1H), 3.52–3.61 (m, 2H), 3.71–3.90 (m, 3H), 3.93–3.98 (m, 1H), 4.05–4.10 (m, 1H), 4.53–5.01 (m, 8H), 7.25–7.36 (m, 20H); HR-MALDI (positive) calcd m/z for $C_{35}H_{37}O_5Br$: 618.1804. Found: 641.1702, ($M+Na^+$).

(−)-2,4,5,6-Tetra-*O*-benzyl-3-deoxy-3-iodomethyl-*myo*-inositol (20). Removal of the *p*-methoxybenzyl group of (+)-**14** (27 mg, 0.034 mmol) with TFA, as described for **17**, gave (−)-**20** (syrup, 19 mg, 94%); $[\alpha]_D^{20}$ −8.7° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 2.12 (br s, 1H), 2.52–2.57 (m, 1H), 3.56–3.66 (m, 3H), 3.69–3.86 (m, 3H), 4.03–4.07 (m, 1H), 4.44–5.01 (m, 8H), 7.25–7.35 (m, 20H); HR-MALDI (positive) calcd m/z for $C_{35}H_{37}O_5I$: 664.6686. Found: 687.6583 ($M+Na^+$).

(−)-2,4,5,6-Tetra-*O*-benzyl-3-deoxy-3-azidomethyl-*myo*-inositol (21). Removal of the *p*-methoxybenzyl group of (−)-**15** (18 mg, 0.026 mmol) TFA, as described for **17**, gave (−)-**21** (syrup, 14 mg, 94%); $[\alpha]_D^{20}$ −6.3° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 2.42 (br s, 1H), 2.64–2.72 (m, 1H), 3.54–3.65 (m, 1H), 3.72 (t, $J=8.4$ Hz, 1H), 3.85 (t, $J=9.3$ Hz, 1H), 3.91–3.97 (m, 1H), 4.07–4.23 (m, 3H), 4.38–4.59 (m, 4H), 4.69–4.99 (m, 4H), 7.25–7.33 (m, 20H); HR-MALDI (positive) calcd m/z for $C_{35}H_{37}O_5N_3$: 579.2733. Found: 602.2630 ($M+Na^+$).

(+)-2,4,5,6-Tetra-*O*-benzyl-3-deoxy-3-benzyloxymethyl-*myo*-inositol (22). Removal of the *p*-methoxybenzyl group of (−)-**16** (35 mg, 0.046 mmol) TFA, as described for **17**, gave (+)-**22** (syrup, 27 mg, 92%); $[\alpha]_D^{20}$ +6.0° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 2.17 (s, 1H), 2.59–2.68 (m, 1H), 3.34 (t, $J=9.9$ Hz, 1H), 3.44–3.71 (m, 4H), 3.94–4.09 (m, 2H), 4.33–4.61 (m, 6H), 4.70–5.02 (m, 4H), 7.26–7.38 (m, 25H); HR-MALDI (positive) calcd m/z for $C_{42}H_{44}O_6$: 644.3138. Found: 667.3036 ($M+Na^+$).

(+)-1-*O*-(1,2-Di-*O*-palmitoyl-*sn*-glycerol-3-benzoxyphosphoryl)-2,4,5,6-tetra-*O*-benzyl-3-deoxy-3-fluoromethyl-*myo*-inositol (24). A solution of 1,2-di-*O*-palmitoyl-*sn*-glycerol 3-(benzyl *N*,*N*-diisopropylphosphoramidite) **23** (73 mg, 0.047 mmol) and 1*H*-tetrazole (10 mg, 0.063 mmol) in CH_2Cl_2 (2 mL) was stirred at 23 °C under argon for 30 min and (−)-**17** (17 mg, 0.031 mmol) was added. The mixture was kept under the same conditions for another 12 h, cooled to −40 °C, and then treated with *m*-CPBA (19 mg, 57% purity, 0.064 mmol). The reaction mixture was stirred at −40 °C for 30 min, allowed to attain room temperature for 1 h, diluted with CH_2Cl_2 (20 mL), washed, in tandem, with 10% $NaHCO_3$ solution and brine, dried, and concentrated. Column chromatography (ether–hexane 1:3) of the residue gave (+)-**24** (syrup, 33.6 mg, 85%); $[\alpha]_D^{20}$ +2.4° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.87 (t, $J=7.2$ Hz, 6H), 1.20–1.30 (m, 48H), 1.42–1.60 (m, 4H), 2.20–2.30 (m, 5H), 3.45–3.52 (m, 1H), 3.85–4.10 (m, 9H), 4.20–4.25 (m, 1H), 4.47–4.93 (m, 10H), 4.96–5.05 (m, 1H), 7.24–7.34 (m, 25H); ^{31}P NMR ($CDCl_3$, H_3PO_4 as external reference) δ −1.42, −1.51; HR-MALDI (positive) calcd m/z for $C_{77}H_{111}O_{12}FP$: 1277.7797. Found: 1300.6795 ($M+Na_+$).

(+)-1-*O*-(1,2-Di-*O*-palmitoyl-*sn*-glycerol-3-benzoxyphosphoryl)-2,4,5,6-tetra-*O*-benzyl-3-deoxy-3-chloromethyl-*myo*-inositol (25). Coupling of (−)-**18** (18 mg, 0.031 mmol) with **23** (73 mg, 0.047 mmol), as described for **24**, yielded (+)-**25** (syrup, 33 mg, 80%); $[\alpha]_D^{20}$ +10.0° (*c* 2, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.87 (t, $J=7.2$ Hz, 6H), 1.20–1.32 (m, 48H), 1.48–1.64 (m, 4H), 2.17–2.25 (m, 4H), 2.54–2.62 (m, 1H), 3.41 (t, $J=11.4$ Hz, 1H), 3.51–3.60 (m, 2H), 3.76–3.81 (m, 1H), 3.92–4.19 (m, 6H), 4.29 (t, $J=7.8$ Hz, 1H), 4.39–4.71 (m, 5H), 4.79–5.08 (m, 6H), 7.25–7.36 (m, 25H); ^{31}P NMR ($CDCl_3$, H_3PO_4 as external reference) δ −1.12, −1.37; HR-MALDI (positive) calcd m/z for $C_{77}H_{111}O_{12}ClP$: 1293.7502. Found: 1316.3399 ($M+Na^+$).

(+)-1-*O*-(1,2-Di-*O*-palmitoyl-*sn*-glycerol-3-benzoxyphosphoryl)-2,4,5,6-tetra-*O*-benzyl-3-deoxy-3-bromomethyl-*myo*-inositol (26). Coupling of (−)-**19** (20 mg, 0.032 mmol) with **23** (73 mg, 0.047 mmol), as described for **24**, yielded (+)-**26** (syrup, 35 mg, 81%); $[\alpha]_D^{20}$ +8.2° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.87 (t, $J=7.2$ Hz, 6H), 1.22–1.28 (m, 48H), 1.48–1.60 (m, 4H), 2.18–2.25 (m, 4H), 2.56–2.60 (m, 1H), 3.28 (t, $J=9.9$ Hz, 1H), 3.44–3.56 (m, 2H), 3.65–3.77 (m, 2H), 3.96–4.30 (m, 6H), 4.40–4.70 (m, 4H), 4.78–5.11 (m, 7H), 7.24–7.34 (m, 25H); ^{31}P NMR ($CDCl_3$, H_3PO_4 as external reference) δ −1.13, −1.37; HR-MALDI (positive) calcd m/z for $C_{77}H_{111}O_{12}BrP$: 1337.6997. Found 1360.6894 ($M+Na^+$).

(+)-1-*O*-(1,2-Di-*O*-palmitoyl-*sn*-glycerol-3-benzoxyphosphoryl)-2,4,5,6-tetra-*O*-benzyl-3-deoxy-3-iodomethyl-*myo*-inositol (27). Coupling of (−)-**20** (18 mg, 0.027 mmol) with **23** (73 mg, 0.047 mmol), as described for **24**, yielded (+)-**27** (syrup, 32 mg, 85%); $[\alpha]_D^{20}$ +8.7° (*c* 2, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.87 (t, $J=7.2$ Hz, 6H), 1.18–1.36 (m, 48H), 1.44–1.62 (m, 4H), 2.16–2.24 (m, 4H), 2.68–2.76 (m, 1H), 3.47–3.50 (m, 1H), 3.60–3.68 (m, 2H), 3.92–4.31 (m, 8H), 4.47–5.18 (m, 11H), 7.25–7.30 (m, 25H); ^{31}P NMR ($CDCl_3$, H_3PO_4 as external reference) δ −1.12, −1.37; HR-MALDI (positive) calcd m/z for $C_{77}H_{111}O_{12}IP$: 1385.6858. Found: 1408.6756 ($M+Na^+$).

(+)-1-*O*-(1,2-Di-*O*-palmitoyl-*sn*-glycerol-3-benzoxyphosphoryl)-2,4,5,6-tetra-*O*-benzyl-3-deoxy-3-azidomethyl-*myo*-inositol (28). Coupling of (−)-**21** (13 mg, 0.023 mmol) with **23** (54 mg, 0.035 mmol), as described for **24**, yielded (+)-**28** (24 mg, 83%); $[\alpha]_D^{20}$ +8.2° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.87 (t, *J* = 7.2 Hz, 6H), 1.20–1.30 (m, 48H), 1.46–1.67 (m, 4H), 2.13–2.24 (m, 4H), 2.60–2.68 (m, 1H), 3.56–3.65 (m, 2H), 3.95–4.21 (m, 7H), 4.29–4.48 (m, 2H), 4.52–5.05 (m, 11H), 7.25–7.38 (m, 25H); ^{31}P NMR ($CDCl_3$, H_3PO_4 as external reference) δ −0.57; HR-MALDI (positive) calcd *m*/*z* for $C_{77}H_{111}O_{12}N_3P$: 1300.7905. Found 1323.7802 ($M+Na^+$).

(+)-1-*O*-(1,2-Di-*O*-palmitoyl-*sn*-glycerol-3-benzoxyphosphoryl)-2,4,5,6-tetra-*O*-benzyl-3-deoxy-3-benzyloxymethyl-*myo*-inositol (29). Coupling of (−)-**22** (24 mg, 0.037 mmol), with **23** (87 mg, 0.056 mmol), as described for **24**, yielded (+)-**29** (syrup, 41 mg, 81%); $[\alpha]_D^{20}$ +11.0° (*c* 2, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.87 (t, *J* = 7.2 Hz, 6H), 1.19–1.36 (m, 48H), 1.45–1.62 (m, 4H), 2.17–2.38 (m, 4H), 2.58–2.65 (m, 1H), 3.46–3.65 (m, 3H), 3.98–4.29 (m, 7H), 4.33–4.52 (m, 1H), 4.48–5.05 (m, 13H), 7.25–7.36 (m, 30H); ^{31}P NMR ($CDCl_3$, H_3PO_4 as external reference) δ −0.46; HR-MALDI (positive) calcd *m*/*z* for $C_{84}H_{118}O_{13}P$:1365.8310. Found: 1388.8207 ($M+Na^+$).

L-α-Phosphatidyl-D-3-deoxy-3-fluoromethyl-*myo*-inositol (1). A suspension of **24** (32 mg, 0.025 mmol) and palladium black (30 mg) in aqueous 80% ethanol (4 mL) was shaken under H_2 (55 psi) for 16 h, filtered and concentrated. The residual aqueous solution was lyophilized to furnish **1** (lyophilized powder, 20 mg, 97%); $[\alpha]_D^{20}$ +9.1° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.87 (t, *J* = 7.2 Hz, 6H), 1.20–1.32 (m, 48H), 1.49–1.64 (m, 4H), 2.23–2.40 (m, 4H), 3.44–3.60 (m, 1H), 3.72–3.73 (m, 1H), 3.95–4.29 (m, 10H), 5.08–5.30 (m, 1H); ^{31}P NMR ($CDCl_3$, H_3PO_4 as external reference) δ −1.01; HR-MALDI (negative) calcd *m*/*z* for $C_{42}H_{81}O_{12}FP$: 827.5450. Found: 807.5387 (M−HF), 850.5347 ($M+Na^+$).

L-α-Phosphatidyl-D-3-deoxy-3-chloromethyl-*myo*-inositol (2). The perbenzylated derivative (+)-**25** (31 mg, 0.024 mmol) was subjected to hydrogenolysis, as described for **24**, gave **2** (lyophilized powder, 19 mg, 94%); $[\alpha]_D^{20}$ +2.8° (*c* 2, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.87 (t, *J* = 7.2 Hz, 6H), 1.21–1.35 (m, 48H), 1.49–1.64 (m, 4H), 2.28–2.37 (m, 4H), 3.40–4.45 (m, 12H), 5.20–5.30 (m, 1H); ^{31}P NMR ($CDCl_3$, H_3PO_4 as external reference) δ −1.22; HR-MALDI (negative) calcd *m*/*z* for $C_{42}H_{81}O_{12}ClP$: 843.5155. Found: 807.5387 (M−HCl), 866.5053 ($M+Na^+$).

L-α-Phosphatidyl-D-3-deoxy-3-bromomethyl-*myo*-inositol (3). The perbenzylated derivative (+)-**26** (33 mg, 0.025 mmol) was subjected to hydrogenolysis, as described for **24**, gave **3** (lyophilized powder, 21 mg, 96%); $[\alpha]_D^{20}$ +6.3° (*c* 2, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.87 (t, *J* = 7.2 Hz, 6H), 1.21–1.32 (m, 48H), 1.50–1.58 (m, 4H), 2.29–2.37 (m, 4H), 3.18–3.77 (m, 4H), 3.92–4.56 (m, 8H), 5.20–5.30 (m, 1H); ^{31}P NMR ($CDCl_3$, H_3PO_4 as external reference) δ −1.03; HR-MALDI (negative) calcd *m*/*z* for $C_{42}H_{81}O_{12}BrP$: 889.4629. Found: 807.5387 (M−HBr).

L-α-Phosphatidyl-D-3-deoxy-3-iodomethyl-*myo*-inositol (4). The perbenzylated derivative (+)-**27** (30 mg, 0.022 mmol) was subjected to hydrogenolysis, as described for **24**, gave **4** (lyophilized powder, 19 mg, 94%); $[\alpha]_D^{20}$ +3.0° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.87 (t, *J* = 7.2 Hz, 6 H), 1.21–1.35 (m, 48H), 1.48–1.62 (m, 4H), 2.28–2.37 (m, 4H), 3.47–3.51 (m, 1H), 3.66–3.67 (m, 1H), 3.72–3.77 (m, 1H), 4.08–4.30 (m, 9H), 5.20–5.30 (m, 1H); ^{31}P NMR ($CDCl_3$, H_3PO_4 as external reference) δ −0.86; HR-MALDI (negative) calcd *m*/*z* for $C_{42}H_{81}O_{12}IP$: 934.4432. Found: 807.5387 (M−HI).

L-α-Phosphatidyl-D-3-deoxy-3-aminomethyl-*myo*-inositol (5). The perbenzylated derivative (+)-**28** (22 mg, 0.017 mmol) was subjected to hydrogenolysis, as described for **24**, gave **5** (lyophilized powder, 13.2 mg, 92%); $[\alpha]_D^{20}$ +3.8° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.87 (t, *J* = 7.2 Hz, 6H), 1.21–1.25 (m, 48H), 1.49–1.62 (m, 4H), 2.25–2.40 (m, 4H), 3.46–4.30 (m, 12H), 4.60–4.70 (m, 1H), 5.20–5.30 (m, 1H); ^{31}P NMR ($CDCl_3$, H_3PO_4 as external reference) δ −1.11; HR-MALDI (negative) calcd *m*/*z* for $C_{42}H_{83}O_{12}NP$: 824.5653. Found: 823.5575 (M−H).

L-α-Phosphatidyl-D-3-deoxy-3-hydroxymethyl-*myo*-inositol (6). The perbenzylated derivative (+)-**29** (38 mg, 0.028 mmol) was subjected to hydrogenolysis, as described for **24**, gave **6** (lyophilized powder, 22 mg, 95%); $[\alpha]_D^{20}$ +6.9° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.87 (t, *J* = 7.2 Hz, 6H), 1.25–1.37 (m, 48H), 1.50–1.67 (m, 4H), 2.17–2.38 (m, 4H), 3.45–4.51 (m, 1H), 3.60–4.48 (m, 11H), 5.20–5.30 (m, 1H); ^{31}P NMR ($CDCl_3$, H_3PO_4 as external reference) δ −0.99; HR-MALDI (negative) calcd *m*/*z* for $C_{42}H_{82}O_{13}P$: 825.5493. Found 824.5415 (M−H), 848.5391, ($M+Na^+$).

PI 3-kinase assay

The enzyme assay was carried out using PI 3-kinase that was immunoprecipitated with anti-p85 antibodies from rat liver cytosol as described.[23] In brief, PI(4,5)P_2 (10 μg) and phosphatidylserine (40 μg) were suspended in 100 μL of 30 mM Hepes, pH 7.5, containing 1 mM EDTA and 1 mM EGTA, sonicated in a water bath-type sonicator for 5 min, and mixed vigorously with a vortex mixer before assays. The immunoprecipitated PI 3-kinase in 30 mM Hepes, pH 7.5, with appropriate dilution (10 μL) was incubated with 80 μL of the same buffer containing 125 μM ATP, 10 μCi [γ-^{32}P]ATP, and 6.25 mM $MgCl_2$. The reaction was initiated by adding 10 μL of the phospholipid solution, incubated at 37 °C for 10 min, and stopped by adding 5 μL of 1 mM EDTA and 25 μL of 5 M HCl, followed by 160 μL of $CHCl_3$–CH_3OH (1:1). The phases were separated by centrifugation at 6000*g* for 5 min. The organic layer was dried by a stream of N_2, spotted onto 1% oxalic acid-treated TLC plates, and then developed with *n*-propranol–2M acetic acid (65:35) overnight. After drying, spots were located by autoradiography and compared

with standards. The autoradiograms were scanned by a Photodyne image system and quantified using an NIH imaging program (version 1.59).

Hexosaminidase secretion assay

The release of mast cell mediators by exocytosis was monitored by the β-hexosaminidase assay. RBL-2H3 cells were grown in 12-well plates and passively sensitized with DNP-specific IgE. The IgE-sensitized cells were washed twice with Tyrode's buffer consisting of 10 mM Hepes, pH 7.4, 130 mM NaCl, 5 mM KCl, 1.4 mM $CaCl_2$, 1 mM $MgCl_2$, 5.6 mM glucose, and 0.1% BSA. Secretion was initiated by exposing cells to the antigen DNP-HSA (1 μg/mL). After 1 h, the reaction was terminated by placing the plate on ice. The enzyme activities of β-hexosaminidase in 50 μL of supernatants and attached cells solubilized with 0.5% Triton X-100 were measured with 200 μL of 1 mM *p*-nitrophenyl *N*-acetyl-β-D-glucosaminide in 0.1 M sodium citrate, pH 4.5, at 37 °C for 1 h. The reaction was stopped by the addition of 500 μL of 0.1 M $NaHCO_3$. The release of the product *p*-nitrophenol was measured by monitoring the absorbance at 400 nm. Percentage of degranulation was calculated by dividing the absorbance of the supernatant over the combined absorbance of the supernatant and cell lysate.

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