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Synthesis and Evaluation of Lysophosphatidylserine Analogues as Inducers of Mast Cell Degranulation. Potent Activities of Lysophosphatidylthreonine and Its 2-Deoxy Derivative

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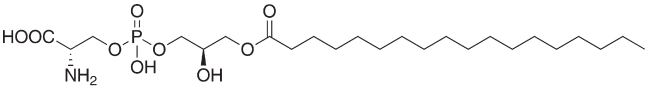
In response to various exogenous stimuli, mast cells (MCs) release a wide variety of inflammatory mediators stored in their cytoplasmic granules and this release initiates subsequent allergic reactions. Lysophosphatidylserine (lysoPS) has been known as an exogenous inducer to potentiate histamine release from MCs, though even at submicromolar concentrations. In this study, through SAR studies on lysoPS against MC degranulation, we identified lysoPT, a threonine-containing lysophospholipid and its 2-deoxy derivative as novel strong agonists. LysoPT and its 2-deoxy derivative induced histamine release from MCs both in vitro and in vivo at a concentration less than one-tenth that of lysoPS. Notably, lysoPT did not activate a recently proposed lysoPS receptor on MCs, GPR34, demonstrating the presence of another undefined receptor reactive to both lysoPS and lysoPT that is involved in MC degranulation. Thus, the present strong agonists, lysoPT and its 2-deoxy derivative, will be useful tools to understand the mechanisms of lysoPS-induced activation of degranulation of MCs.

Introduction

Mast cells (MCs[a]) play an important role in immediate-type allergic reactions by releasing chemical mediators such as histamine and serotonin from their secretary granules in response to an antigen.[1,2] Histamine release from MCs is a key process in allergic diseases such as pollinosis, urticaria, atopic dermatitis, and asthma. Indeed, more than half of the population suffers from such diseases, especially in developed countries, and the number of patients is increasing annually. Identification of molecules that modulate MC degranulation would be helpful in providing tools to investigate the molecular mechanisms of allergic reactions as well as candidate antiallergy drugs.

Exogenous lysophosphatidylserine (lysoPS; *sn*-1-acyl-lysoPS) has been known to strongly enhance the degranulation of rodent MCs.[3] Upon cross-linking of IgE-bound FcεRI (a high-affinity receptors for IgE) on peritoneal MCs from rodents by antigens or concanavalin A (ConA), a cross-linker of FcεRI, lysoPS significantly enhances the degranulation responses.[3,4] LysoPS also induces the degranulation of peritoneal MCs when injected i.v. in rodents even in the absence of FcεRI-cross-linking. Enhancement of MC degranulation, however, can be observed at a rather high dose, i.e., a submicromolar concentration of lysoPS. In addition, the action is highly specific to lysoPS, and all other lysophospholipids examined, including the analogue containing the unnatural D-serine moiety, were ineffective inducers of MC degranulation.[7]



Lysophosphatidylserine (LysoPS (18:0))

Thus, a specific receptor for lysoPS has been postulated to be present on MCs.[5,6,11] It is likely that lysoPS is produced from phosphatidylserine (PS), which is exposed on the cell surface of apoptotic or activated cells, by the action of PS-specific phospholipase A_1 (PS-PLA_1).[8−10] Recently, Sugo et al. reported that an orphan G-protein-coupled receptor, called GPR34, was activated specifically by lysoPS.[11] Because GPR34 was expressed in MC, they proposed that GPR34 was a strong candidate for the putative lysoPS receptor on MCs.[11]

While the enhancement of the MC degranulation is highly specific to lysoPS among other lysophospholipids,[7] the effective concentration of lysoPS is rather high, so that it may

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[a] Abbreviations: MCs, mast cells; lysoPS, lysophosphatidylserine; lysoPT, lysophosphatidylthreonine; PS, phosphatidylserine; ConA, concanavalin A; FcεRI, a high-affinity receptors for IgE; anti-DNP-AS, antidinitrophenol-ascaris antibody; BMMCs, bone marrow-derived mast cells; LDH, lactate dehydrogenase; BSA, bovine serum albumin; RPMCs, rat peritoneal mast cells; MPMCs, mouse peritoneal mast cells; Bn, benzyl; Z, benzyloxycarbonyl; DEAD, diethyl azodicarboxylate; CSA, camphorsufonic acid; MMTr, 4-methoxytrityl; DIPEA, diisopropylethylamine; TBAI, tetrabutylammonium iodide; *p*-TSA, *p*-toluenesulfonic acid; DMAP, *N*,*N*-dimethylaminopyridine; CAN, cerium(IV) ammonium nitrate.

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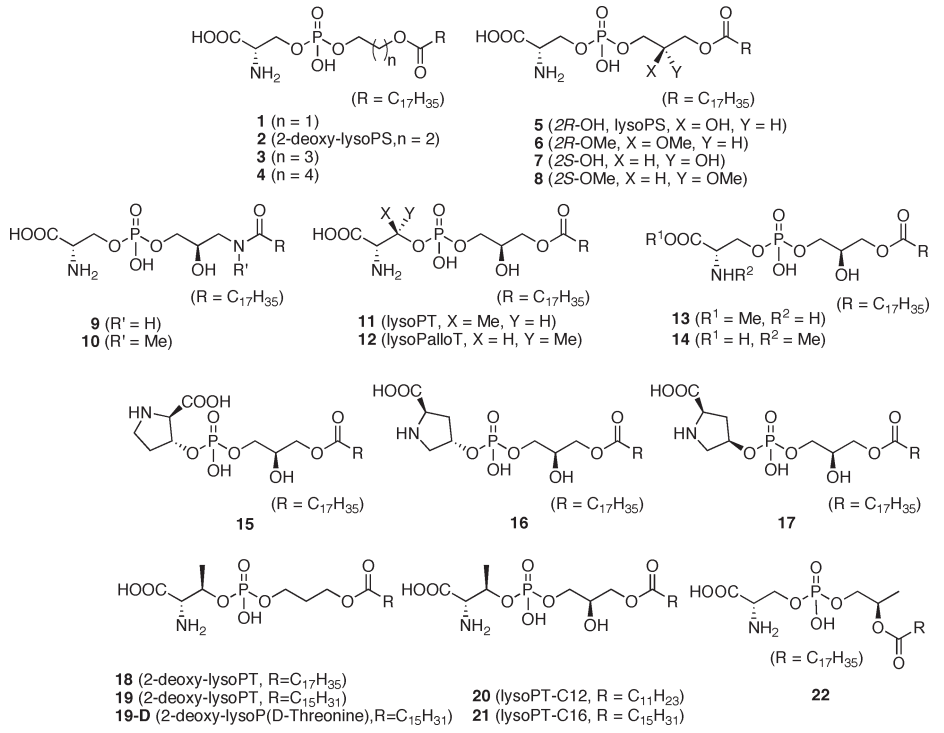
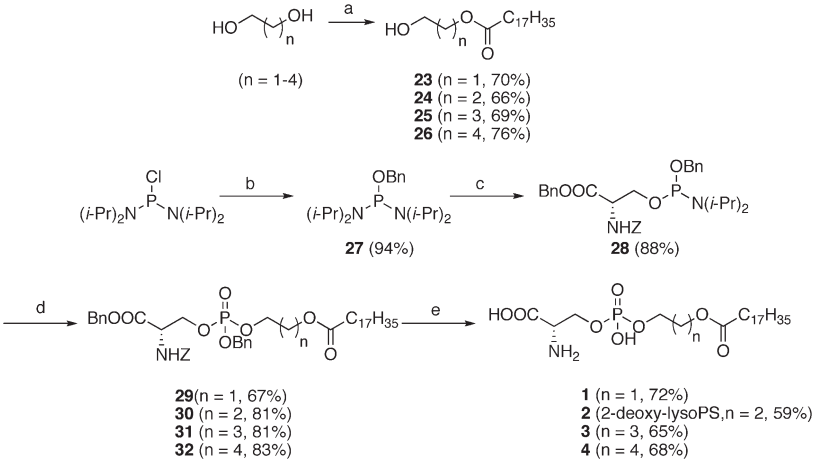


Figure 1. LysoPS analogues synthesized in this study.

Scheme 1. Synthesis of **1**−**4**[a]



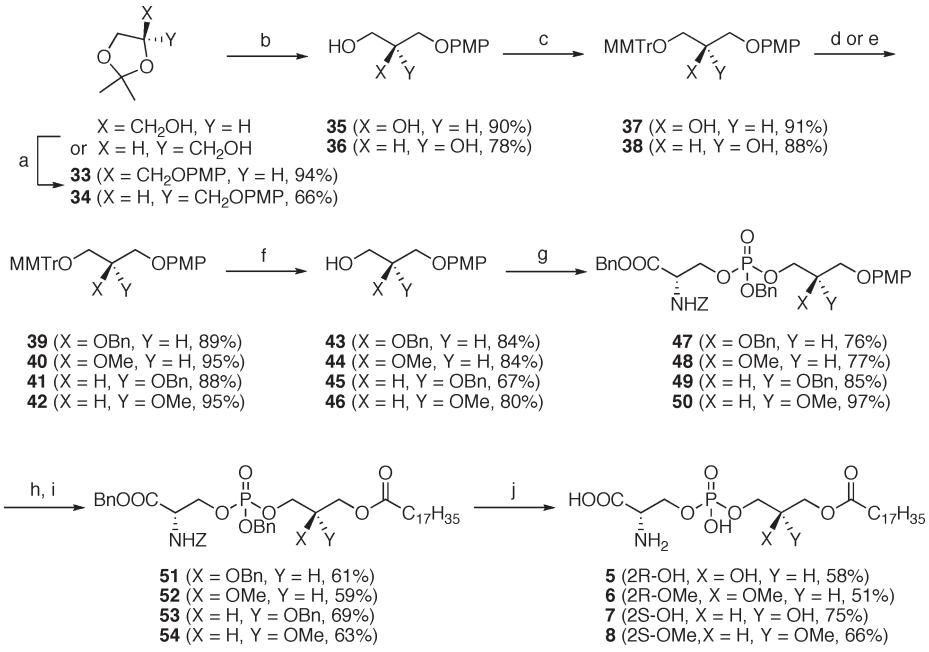
[a] (a) Stearoyl chloride, pyridine, CH_2Cl_2, rt, 1 d; (b) BnOH, Et_3N, Et_2O, 0 °C, 2 h; (c) *N*-Z-L-Ser benzyl ester, 1*H*-tetrazole, CH_2Cl_2-THF, rt, 2 h; (d) **23**−**26**, 1*H*-tetrazole, CH_2Cl_2-THF, rt, 2 h, then TBHP, rt, 1 h; (e) Pd−C (10%), H_2, MeOH-AcOH, rt, 1 d.

be possible to design higher-affinity agonistic ligands. Such high-affinity ligands would be useful tools for mechanistic studies of MC degranulation. In this study, we therefore synthesized a number of lysoPS analogues and finally found that simple replacement of the serine residue of lysoPS with the threonine residue (i.e., threonine-containing lysophospholipid (lysophosphatidylthreonine, lysoPT) and its 2-deoxy derivative) resulted in a dramatic increase in potency for inducing MC degranulation and in vivo hypothermic effects. This in vivo hypothermic effect was not detected in MC-deficient mice. This fact strongly supported the postulate that these new derivatives also target MCs in vivo. We disclosed here the structure−activity relationships of the chemical inducers of MC degranulation. In addition, using the present agonist, lysoPT, we demonstrated that GPR34 is not activated by lysoPT, suggesting a postulate that GPR34 is not the common receptor involved in the MC degranulation.

Chemistry

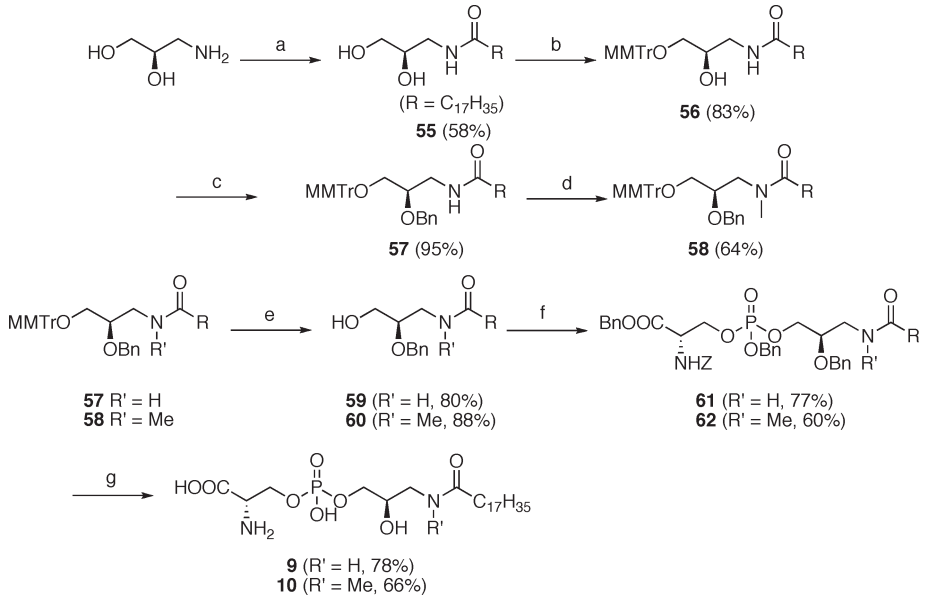
We synthesized a variety of lysoPS analogues (**1**−**22**), including lysoPS (**5**) itself (Figure 1; for details of the synthesis and characterization of lysoPS analogues (**1**−**22**), see the Experimental Section and Schemes 1−9). In designing these molecules, we modified the glycerol part (**1**−**8**) (Schemes 1 and 2), the fatty acid linkage (**9** and **10**) (Scheme 3), added a methyl group at carbon and hetero atoms of various positions of the serine moiety (methyl scanning, **11**−**14**) (Schemes 4 and 5), and conformationally constrained the serine moiety (**15**−**17**) (Scheme 6). Because the chain length of the fatty acid moiety might affect the potency of lysoPS analogues, a stearic acid (18:0) unit was principally used in this

Scheme 2. Synthesis of **5**−**8**[a]



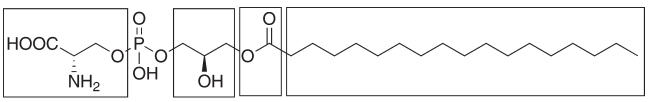
[a] (a) *p*-Methoxyphenol, DEAD, PPh_3, toluene, 70 °C, 2 h; (b) CSA, MeOH, rt, 1 d; (c) MMTrCl, DIPEA, TBAI, CH_2Cl_2, rt, 2 h; (d) BnBr, NaH, TBAI, THF, 0 °C, 10 min, to rt, 1 h; (e) MeI, NaH, THF, 0 °C 10 min to rt, 2 h; (f) CSA or *p*-TSA, MeOH-CH_2Cl_2 (3:1), 30 min; (g) **28**, 1*H*-tetrazole, CH_2Cl_2-THF, rt, 2 h, then TBHP, rt, 1 h; (h) CAN, CH_3CN-H_2O (4:1), 0 °C, 20 min; (i) stearoyl chloride, DMAP, CH_2Cl_2, 0 °C to rt, 30 min; (j) Pd−C (10%), H_2, MeOH-AcOH, rt, 1 d.

Scheme 3. Synthesis of **9** and **10**[a]



[a] (a) Stearoyl chloride, pyridine, DMF, 50 °C, 3 h; (b) MMTrCl, DIPEA, TBAI, THF, 50 °C, 2 h; (c) BnBr, NaH, THF, 0 °C, 10 min, to rt, 1 h; (d) MeI, NaH, THF, 0 °C 10 min to rt, 2 h; (e) CSA, MeOH-CH_2Cl_2 (3:1), 30 min; (f) **28**, 1*H*-tetrazole, CH_2Cl_2-THF, rt, 2 h, then TBHP, rt, 1 h; (g) Pd−C (10%), H_2, MeOH-AcOH, rt, 1 d.

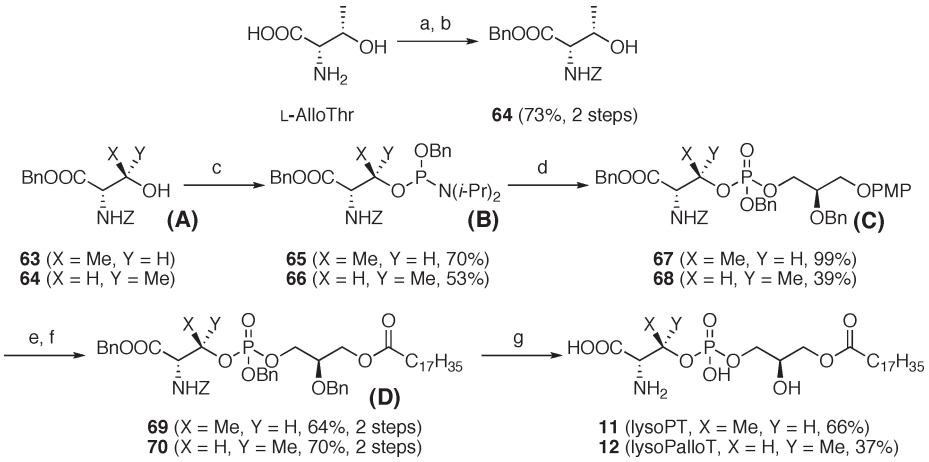
study unless otherwise mentioned. A phosphoramidite method was used to construct the phosphate diester linkage in these analogues.



amino acid part **glycerol part** **fatty acid linkage** **acyl chain**

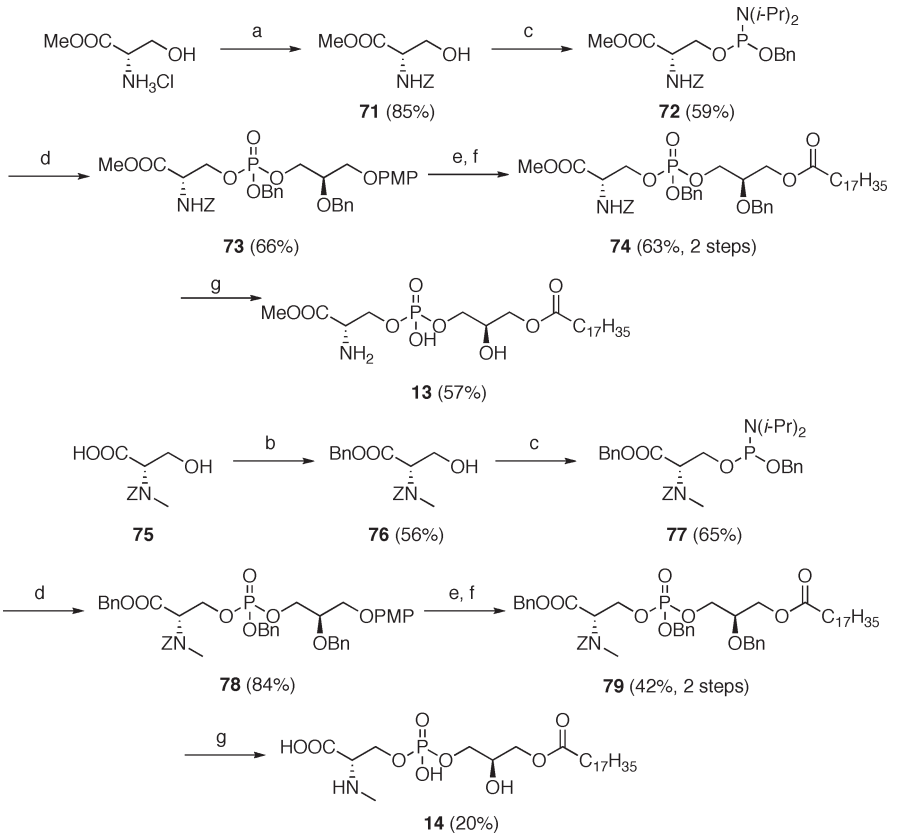
The synthesis of lysoPT (**11**) is shown in Scheme 4. The C, N-terminally protected threonine (**A**) was phosphorylated with benzyloxybis(diisopropylamino)phosphine in the presence of 1*H*-tetrazole (**B**), followed by a second phosphorylation with a protected glycerol.[12] Subsequent in situ oxidation of the resultant phosphite triester intermediate with *tert*-butyl hydroperoxide afforded the fully protected phosphate triester (**C**). Selective oxidative deprotection of the terminal oxygen atom of the glycerol moiety, followed by acylation with stearoyl chloride (**D**), and complete deprotection furnished **11**. We also synthesized the corresponding deoxy derivative of lysoPT, i.e., 2-deoxy-lysoPT (**18** and **19**), as illustrated in Scheme 7.

Scheme 4. Synthesis of **11** and **12**[a]



[a] (a) Z-Cl, sat $NaHCO_3$, THF, 0 °C, 1 h; (b) BnBr, TBAI, THF, rt, 16 h; (c) **27**, 1*H*-tetrazole, CH_2Cl_2-THF, rt, 2 h; (d) **43**, 1*H*-tetrazole, CH_2Cl_2-THF, rt, 2 h, then TBHP, rt, 1 h; (e) CAN, CH_3CN-H_2O (4:1), 0 °C, 20 min; (f) stearoyl chloride, DMAP, CH_2Cl_2, 0 °C to rt, 30 min; (g) Pd−C (10%), H_2, MeOH-AcOH, rt, 1 d.

Scheme 5. Synthesis of **13** and **14**[a]



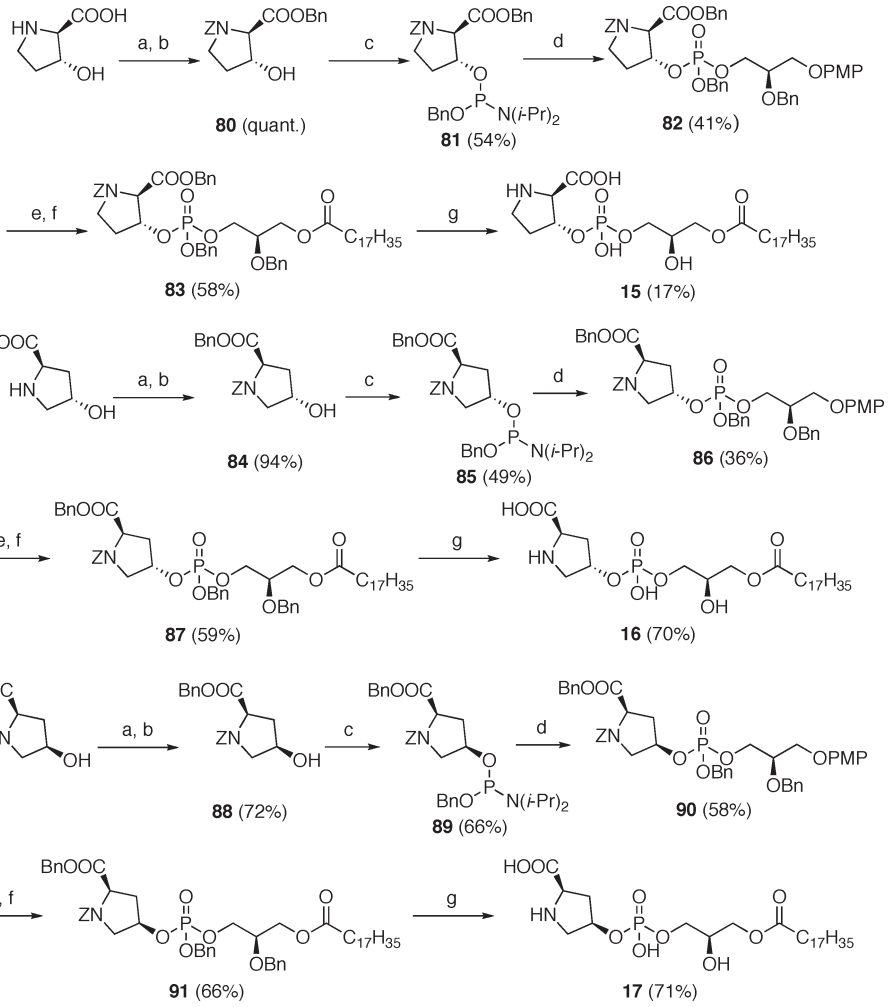
[a] (a) Z-Cl, saturated $NaHCO_3$, THF, 0 °C, 1 h; (b) BnBr, TBAI, THF, rt, 16 h; (c) **27**, 1*H*-tetrazole, CH_2Cl_2-THF, rt, 2 h; (d) **43**, 1*H*-tetrazole, CH_2Cl_2-THF, rt, 2 h, then TBHP, rt, 1 h; (e) CAN, CH_3CN-H_2O (4:1), 0 °C, 20 min; (f) stearoyl chloride, DMAP, CH_2Cl_2, 0 °C to rt, 30 min; (g) Pd−C (10%), H_2, MeOH-AcOH, rt, 1 d.

The effect of the chain length of the fatty acid moiety of **11** was also studied by synthesizing **20** and **21** with different chain length (C12 and C16, respectively) (Scheme 8). To examine the effect of the position of acyl chains, i.e., *sn*-1-acyl vs *sn*-2-acyl analogue, we synthesized **22** as a *sn*-2-acyl-1-deoxy lysoPS analogue (Scheme 9). We tested the potency of lysoPS analogues to activate MCs both in vitro and in vivo. To evaluate the histamine release from MCs in vitro, MCs isolated from the rat or mouse peritoneal cavity were incubated with each analogue in the presence of ConA, an FcεRI cross-linker. The potency of an analogue was defined as the amount of histamine released as a percent of the total amount of histamine contained in the cells.

Results and Discussions

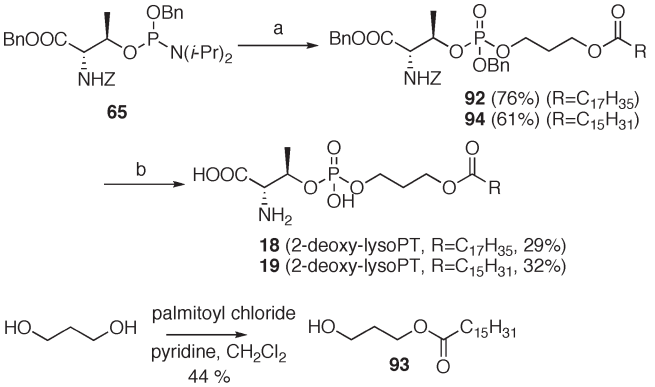
In Vitro Histamine Release from Peritoneal Mast Cells. We examined the effects of the synthetic compounds on the

Scheme 6. Synthesis of **15**−**17**[a]



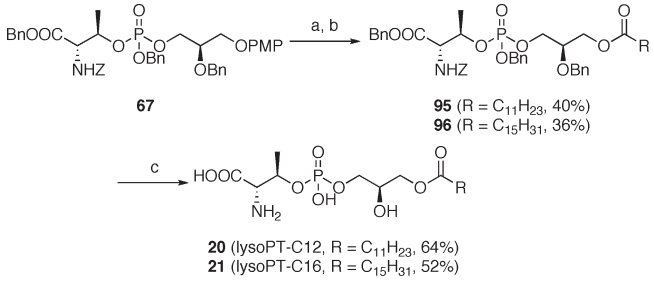
[a] (a) Z-Cl, sat $NaHCO_3$, THF, 0 °C, 1 h; (b) BnBr, TBAI, THF, rt, 16 h; (c) **27**, 1*H*-tetrazole, CH_2Cl_2-THF, rt, 2 h; (d) **43**, 1*H*-tetrazole, CH_2Cl_2-THF, rt, 2 h, then TBHP, rt, 1 h; (e) CAN, CH_3CN-H_2O (4:1), 0 °C, 20 min; (f) stearoyl chloride, DMAP, CH_2Cl_2, 0 °C to rt, 30 min; (g) Pd−C (10%), H_2, MeOH-AcOH, rt, 1 d.

Scheme 7. Synthesis of **18** and **19**[a]



[a] (a) **24** (for **92**) or **93** (for **94**), 1*H*-tetrazole, CH_2Cl_2-THF, rt, 2 h, then TBHP, rt, 1 h; (b) Pd−C (10%), H_2, MeOH-AcOH, rt, 1 d.

Scheme 8. Synthesis of **20** and **21**[a]

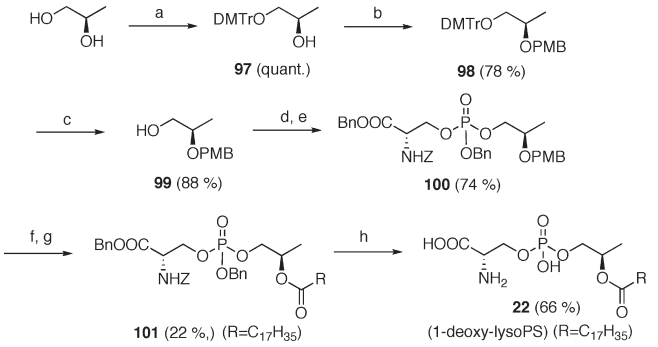


[a] (a) CAN, CH_3CN-H_2O (4:1), 0 °C, 20 min; (b) RCOCl (R=$C_{11}H_{23}$ or $C_{15}H_{31}$), DMAP, CH_2Cl_2, 0 °C to rt, 30 min; (c) Pd−C (10%), H_2, MeOH-AcOH, rt, 1 d.

activation of peritoneal MC degranulation (Table 1, Figure 2, and Figure S1 (Supporting Information)). We first confirmed that the synthetic lysoPS (18:0) (**5**), which has a (*R*)-hydroxyl group at the *sn*-2 position, exhibited degranulation-inducing activity (Figure 2a and 2b). We synthesized lysoPS analogues (**1**−**4** and **6**−**8**), modified at the glycerol part, and some of them were found to retain activity to induce histamine release from rat peritoneal MCs (RPMCs).

For example, **7**, a diastereoisomer bearing an *sn*-2-(*S*)-hydroxyl group, *sn*-2-(*R*)- (**6**), and *sn*-2-(*S*)- (**8**) methyl ether analogues showed activity at concentrations several fold higher than **5** (Table 1 and Figure S1, Supporting Information). The results show that, in contrast to the cases of other lysophospholipids such as lysophosphatidic acid (lysoPA)[13] and their receptors, the *sn*-2 hydroxyl group of

Scheme 9. Synthesis of **22**[a]



[a] (a) DMTrCl, DIPEA, TBAI, CH_2Cl_2, rt; (b)NaH, PMBCl, TBAI, THF, 0−70 °C; (c) PPTS, CH_2Cl_2-MeOH, rt; (d) **28**, 1*H*-tetrazole, CH_2Cl_2-THF; (e) TBHP, rt; (f) DDQ, CH_2Cl_2-phosphate buffer (1:1), 0 °C, 1 h; (g) RCOCl (R=$C_{17}H_{35}$), DMAP, CH_2Cl_2, 0 °C, 1 h; (h) H_2, Pd−C, MeOH, AcOH, rt.

lysoPS is not essential for recognition. This prompted us to synthesize **2**, a deoxy analogue of the *sn*-2-hydroxyl group, i.e., 2-deoxy-lysoPS, because acylation of lysoPS at the *sn*-2 hydroxyl group to regenerate PS is the major inactivation pathway of lysoPS on MCs.[6] It is likely that the 2-deoxy derivative shows superior stability in vivo and in vitro because it is resistant to inactivation by acylation. As expected, **2** was found to show similar activity to **5**, again showing that *sn*-2 hydroxyl group of lysoPS is not essential for its activity (Figure 2a,b and Table 1). We also synthesized *sn*-2-acyl lysoPS analogue, i.e., *sn*-2-acyl-1-deoxy-lysoPS (**22**). Unlike **2**, **22** was found to be inert (data not shown). Thus the position of the *sn*-1-acyl group is crucial in the deoxy derivatives. This is in sharp contrast to the observation of the similar magnitude of the activities of *sn*-1-acyl and *sn*-2-acyl lysoPS on MC degranulation.[8]

Other analogues (**1**, **3**, **4**, **9**, **10**, and **13**−**17**; Table 1 and Figure 1), modified at the glycerol moiety, the fatty acid linkage, and by conformational constriction of the serine moiety, had significantly diminished activity (Figure S1, Supporting Information), indicating that the chain length of the glycerol moiety, the ester linkage at the *sn*-1 position, and the L-serine residue of lysoPS are important for activation of the putative lysoPS receptor.

In the course of methyl scanning of the serine moiety, we found that the synthetic lysoPS analogue that showed the strongest activity in stimulating histamine release was **11**, lysophosphatidylthreonine (lysoPT), which has an additional methyl group at the β-carbon atom of serine in the *R* configuration (L-threonine). Compound **11** induced histamine release from isolated MCs both from rats (Figure 2c) and mice (Figure 2d) at concentrations ~10-fold lower than that of **5**.

Because **2** was found to retain activity, we synthesized the corresponding deoxy derivatives of lysoPT, i.e., 2-deoxy-lysoPT (**18** (stearate) and **19** (palmitate)), as illustrated in Scheme 7. Compounds **18** and **19** showed almost equal activity to **11** in inducing histamine release (Figure 2c,d,e). These results are consistent with the idea that the *sn*-2 hydroxyl group of lysoPS is not essential.

A diastereomer of lysoPT with respect to the stereochemistry of the methyl group, the (*S*)-methyl (i.e., *allo*-threonine) analogue **12**, lysoPalloT (Figure 1), was found to be a poor agonist (Figure 2c,d). 2-Deoxy-lysoPT containing the D-threonine moiety (**19-D**) was ineffective, as expected (Figure 2e). These

Table 1. EC_{50} Values of LysoPS Analogues for Peritoneal Mast Cell Degranulation

compd	EC_{50} rat, μM	EC_{50} mouse, μM
Modification of Glycerol Moiety		
1 (deoxy, n = 1)	>10	nd[a]
2 (2-deoxy-lysoPS, n = 2)	~0.5	~0.04
3 (deoxy, n = 3)	~5	nd
4 (deoxy, n = 4)	>10	nd
5 (lysoPS, 2*R*-OH)	~0.4	~0.1
6 (2*R*-OMe)	~2	nd
7 (2*S*-OH)	~1	~0.07
8 (2*S*-OMe)	~5	nd
Modification of Fatty Acid Linkage		
9 (NH-amide)	~5	nd
10 (NMe-amide)	~10	nd
C-Methyl Scanning		
11 (lysoPT (18:0))	~0.04	~0.01
12 (lysoPalloT)	~2	~0.2
Heteroatom-Methyl Scanning		
13 (lysoPS-CO_2Me)	>10	nd
14 (lysoPS-NHMe)	>10	nd
Conformation-Constrained Serine		
15	>10	nd
16	>10	nd
17	>10	nd
LysoPT Analogues and Others		
18 (2-deoxy-lysoPT (18:0))	~0.1	~0.007
19 (2-deoxy-lysoPT (16:0))	~0.06	nd
19-D (2-deoxy-lysoP(D-T) (16:0))	>10	nd
20 (lyosPT (12:0))	~0.15	~0.05
21 (lysoPT (16:0))	~0.04	~0.02
22 (1-deoxy-lysoPS)	>10	nd

[a] nd = not determined.

results suggest that the stereochemistry of the threonine residue is critically recognized by the putative lysoPS receptor. We confirmed that both **5** and **11** enhanced antigen-induced histamine release from IgE-sensitized RPMCs (Figure 2f). In this case, **11** induced histamine release at a concentration about 10-fold lower than that of **5**.

To examine the effect of acyl chain length in the fatty acid moiety of lysoPT, we synthesized lysoPT analogues derived from lauric acid (12:0) (**20**) or palmitic acid (16:0) (**21**) (Scheme 8). Compound **21** was more potent than **11** in inducing histamine release in vitro, but the activity of **20** was almost equal to that of **11** (Figure 3a,b and Table 1), indicating that hydrocarbon chain length has a significant effect. Compounds **11**, **18**, and **21** were also effective in vivo; they induced rapid elevation of plasma histamine level after i.v. injection of these lysoPT analogues (Figure 3c). The rank order of potencies of lysoPS analogues in MC degranulation was **21** (lysoPT (16:0)) ≫ **11** (lysoPT (18:0)) = **20** (lysoPT (12:0) ≥ **19** (2-deoxy-lysoPT (16:0)) > **18** (2-deoxy-lysoPT (18:0)) > **5** (lysoPS) > **2** (2-deoxy-lysoPS) ≫ **12** (lysoPalloT).

Lysophospholipids may have a nonspecific cell membrane-perturbing, i.e., cytotoxic, effect. Thus, we tested whether or not lysoPT is cytotoxic to MCs by measuring release of a cytoplasmic enzyme, lactate dehydrogenase (LDH). We found that **11**, as well as **5**, did not induce any detectable LDH release even at a high concentration (50 μM), whereas lysophosphatidylcholine (lysoPC), used as

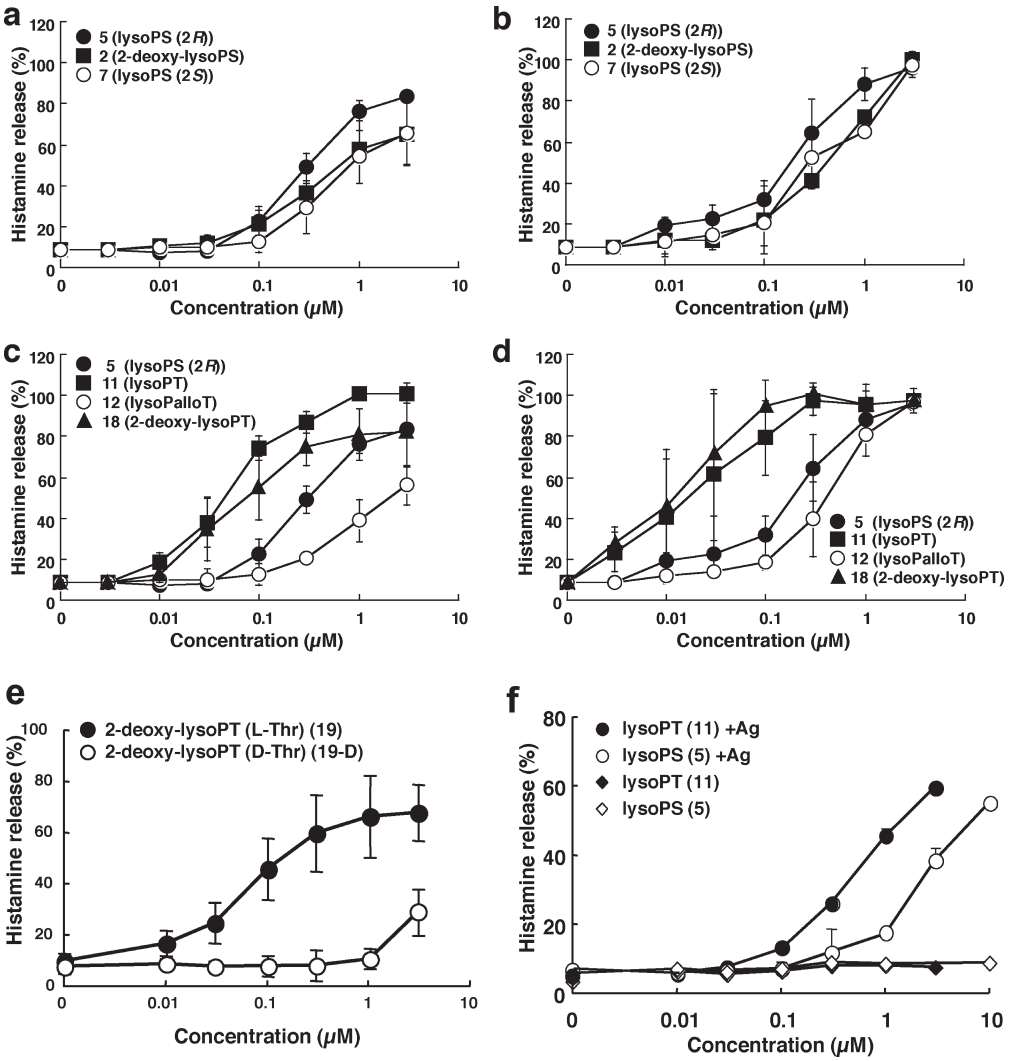


Figure 2. Effect of lysoPS analogues on mast cell degranulation. (a−e) Effect of lysoPS analogues on ConA-induced histamine release from rodent peritoneal mast cells in vitro. Isolated rat (a,c,e) or mouse (b,d) peritoneal mast cells were incubated for 15 min with each lysoPS analogue at the indicated concentrations in the presence of ConA. (f) Effect of lysoPS analogues on antigen-induced histamine release from IgE-sensitized RPMCs in vitro. IgE (anti-DNP-As)-sensitized RPMCs were stimulated with 100 ng/mL DNP-As in the presence of lysoPS (**5**) or lysoPT (**11**). Histamine release was determined by fluorometric assay and is expressed as a percent of the total cell histamine. Values are the means ± SE of three independent experiments.

a cytotoxic positive control, induced significant LDH release (Figure S2, Supporting Information). These data exclude the possibility that lysoPT induces MC degranulation as a result of nonspecific membrane perturbation.

In Vivo Hypothermic Effects of LysoPT and Its 2-Deoxy Derivative. To test the activity of **11** in vivo, we examined whether lysoPT has a hypothermic effect like that induced by lysoPS.[14] When **11** was injected into mice and rats, it caused transient decreases of body temperature at concentrations 20- and 50-fold lower, respectively, than those of **5** (Figure 4a,b,c). As expected, from in vitro experiments (Figure 2), lysoPalloT (**12**) and 2-deoxy-lysoPT containing the D-threonine moiety (**19-D**) were found to be ineffective in inducing the hypothermic effect (data not shown). By contrast, **18** also showed potent hypothermic effects in vivo (Figure 4d). As already mentioned, **11**, **18**, and **21** were effective in vivo to induce rapid elevation of plasma histamine level (Figure 3c).

To confirm that lysoPT really targets MCs in vivo, the hypothermic effects of **11** and **18** were examined in MC-deficient WBB6F1-W/W^v (W/W^v) mice. Compounds **11** (Figure 4e) and **18** (*data not shown*), like **5**,[7] induced no hypothermic response in W/W^v mice, indicating that both **11** and **18** act on MCs.

LysoPT Does Not Activate LysoPS Receptor GPR34. To know possible mechanism of action of lysoPS and lysoPT on MCs, we further examined whether **11** activates GPR34, a recently proposed G-protein-coupled receptor for lysoPS.[11] We confirmed that **5** inhibited forskolin-induced cyclic AMP production (Figure 5a), induced a transient increase in an intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) (Figure 5b), and activated MAP kinase (Figure 5c) in GPR34-expressing CHO-K1 stable transformant (CHO-GPR34) cells. These changes were not observed in mock-transfected CHO-K1 cells. On the other hand, **11** unexpectedly did not induce any of the above three responses in CHO-GPR34 cells (Figure 5a−c). The data clearly show that **11** does not activate GPR34. GPR34 was reported to be highly expressed in bone marrow-derived MCs (BMMCs) in mice. Because BMMCs do not respond to lysoPS,[11] we examined if GPR34 is expressed by lysoPS-reactive peritoneal MCs. As shown in Figure 5d, it was revealed that GPR34 was *not* detected in

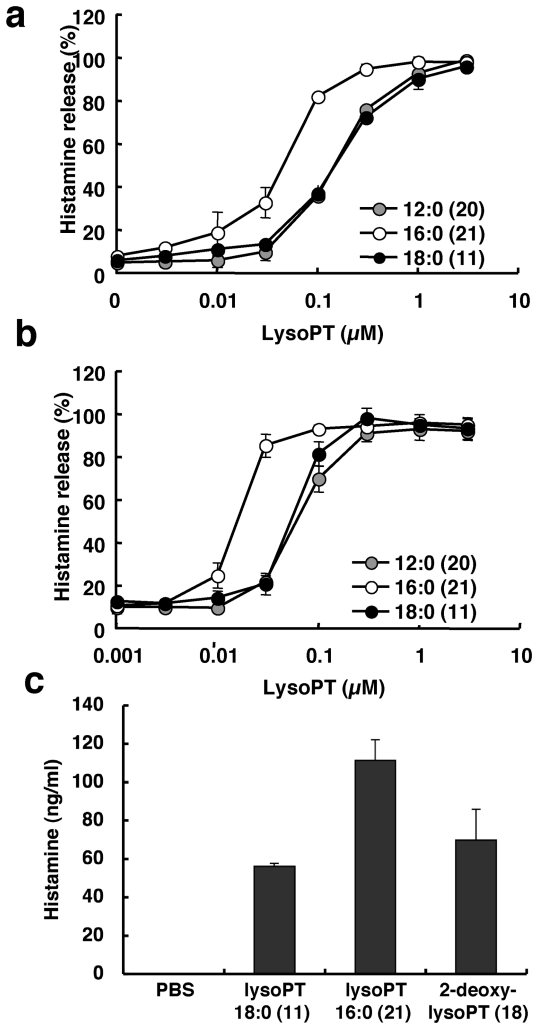


Figure 3. Effect of changing the acyl chain length of lysoPT. (a,b) Rat (a) or mouse (b) peritoneal mast cells were incubated for 15 min with lysoPT containing lauric acid (12:0) (**20**), palmitic acid (16:0) (**21**), or stearic acid (18:0) (**11**) at the indicated concentrations in the presence of 100 μg/mL of ConA. Histamine release is expressed as a percent of the total cell histamine. Values are the means ± SE of three independent experiments. (c) Plasma histamine level of after i.v. injection of lysoPT analogues. C57BL/6 mice were injected intravenously with lysoPT analogues at the indicated dose. Plasma was taken 2 min after the injection, and histamine levels were determined with an EIA system. Values are the means ± SE of three independent experiments.

peritoneal MCs from both mice (Figure 5d) and rats (data not shown), while it was highly expressed by mouse BMMCs as reported previously.[11] All these data indicate that GPR34 is not the receptor involved in lysoPS-induced degranulation in peritoneal MCs. Our results suggest that GPR34 is different from the putative lysoPS receptor responsible for lysoPS-induced degranulation, indicating the presence of novel receptor(s) for lysoPS/lysoPT involved in MC degranulation. So far, the physiological role of GPR34 receptor is not clear. Interestingly, GPR34 was highly expressed in BMMCs, which are regarded as premature MCs. As lysoPS does not stimulate the degranulation of BMMCs, GPR34 may have other function in BMMCs. While the present and past biological data are contradictory, and more biological study will be awaited, the present result demonstrated usefulness of our new agonist (lysoPT) in the relevant research field.

Our present data also suggest that MCs have a mechanism for degranulation that distinguishes the precise and overall structures of **5** and **11**. As shown in Figure S3 of the Supporting Information, RPMCs that were preincubated with **11** and then washed with buffer containing 0.01% BSA (bovine serum albumin) (fatty acid free) still released histamine in response to ConA. In contrast, histamine release from lysoPT-treated MCs was significantly suppressed when the cells were washed with buffer containing 0.5% BSA. Because a high concentration (0.5%) of fatty acid-free BSA is known to extract lysophospholipids from the cell surface, but not from intracellular pools,[6] the result supports the idea that lysoPT binds to its target molecule, probably the putative lysoPT receptor, on the MC surface. Thus, together with several preceding studies, it is strongly suggested that MCs have a target of lysoPT. It remains possible that lysoPS and lysoPT have different individual targets whose activation would induce MC degranulation. However, our experiment using MC-deficient mice confirmed that MCs are the target cells for both lipids, lysoPS and lysoPT (Figure 4e). From the present and past results of the structure−activity relationships, similar modification of **5** and **11** resulted in similar changes in MC degranulation; for example, deoxy-compounds **2** and **18** were both active (Figure 2a−d). Further, both D-serine and D-threonine analogues showed weakened responses (Figure 2e),[7] suggesting that the two lipids target the same molecule on MCs, that is, the putative lysoPS/lysoPT receptor. More biological studies on the mechanisms on lysoPS-induced MC degranulation should be carried out to examine these and other postulations by using the present strong agonists such as **11**, **18**, and **19**.

Conclusion

In the present study, we found strong agonists, **11**, **18**, and **19** as inducers of MC degranulation. The latter 2-deoxy derivarives (**18** and **19**) have an apparent advantage for in vivo/vitro studies because one of the major inactivation processes, i.e., acylation of lysoPS at the *sn*-2 hydroxyl group to regenerate PS, is excluded.

We synthesized a series of novel lysoPS analogues. Most of our synthetic lysoPS analogues with modified serine head groups were inactive, suggesting that the putative lysoPS receptor appears to specifically recognize the structure of lysoPS. Chang et al. previously showed that modification of L-serine part, i.e., D-serine-containing lysoPS (D-lysoPS), dramatically reduced the histamine release-inducing activity.[7] A similar significant loss of activity was observed in the case of 2-deoxy-lysoPT containing the D-threonine moiety, **19-D**. We also found that lysoPS analogues with modifications in glycerol part (**1**, **3**, and **4**) and in acyl chain linkage (**9** and **10**) were found to be inactive (Figure S1, Supporting Information). Thus, the putative lysoPS receptor appears to specifically recognize the structure of lysoPS. However, it is interesting to find that the hydroxyl residue at the *sn*-2 position of lysoPS and lysoPT glycerol backbone did not affect the histamine release-inducing activity, indicating that this residue is not involved in the recognition by the putative lysoPS/lysoPT receptor. The precise mechanism of lysoPS-induced MC degranulation, including the nature of the putative lysoPS/lysoPT receptor, remains to be identified. However, because the receptor is involved in IgE-antigen induced MC degranulation, it is a potential target for antiallergic drugs. Our discovery of the high affinity ligand, lysoPT and 2-deoxy

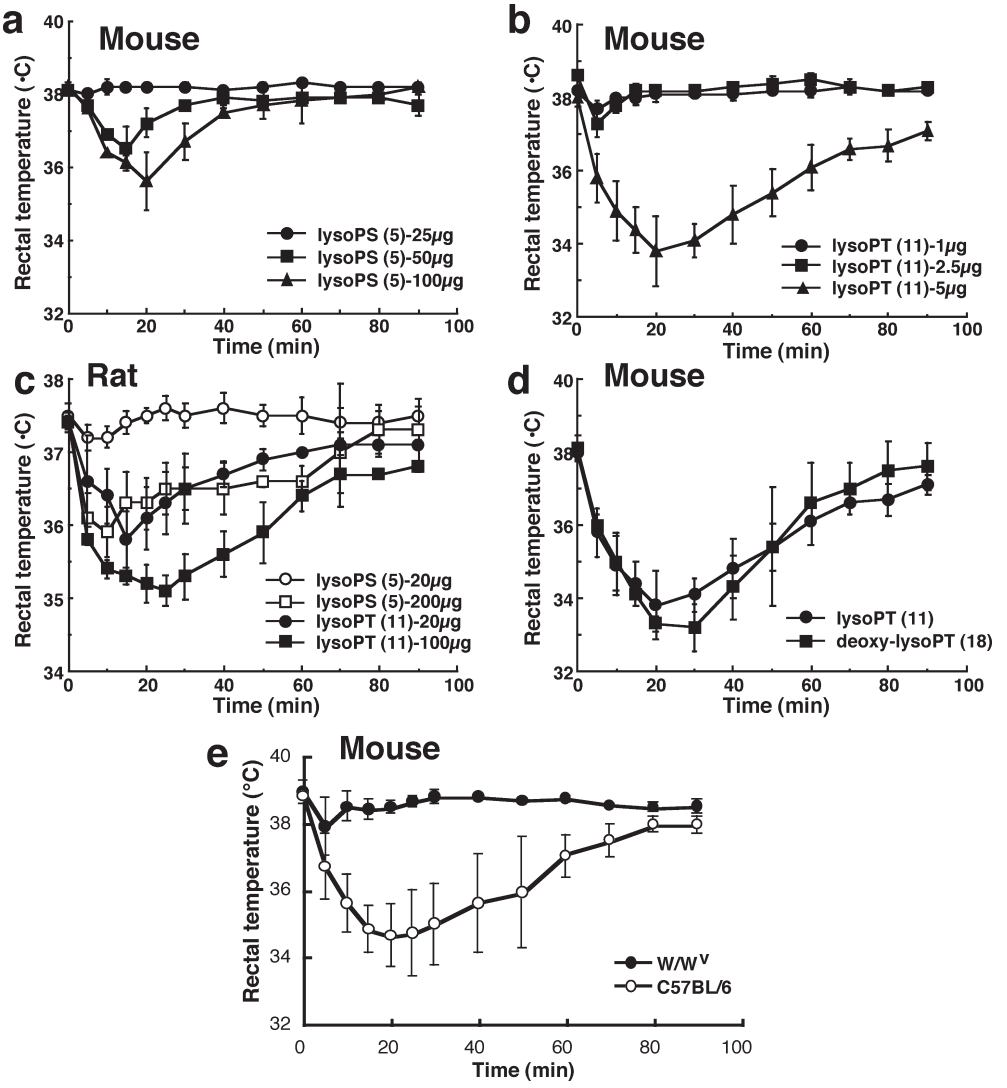


Figure 4. Hypothermic effect of lysoPS analogues. C57BL/6 mice (a,b,d) or Wistar rats (c) were injected intravenously with lysoPS (**5**), lysoPT (**11**), or 2-deoxy-lysoPT (**18**) at the indicated dosage and rectal temperature was monitored every 5 or 10 min. (e) LysoPT does not induce hypothermia in mast cell-deficient (W/W^v) mice. C57BL/6 mice or W/W^v mice were injected i.v. with 10 μg of lysoPT and rectal temperature was monitored. Values are the means ± SE of three independent experiments.

lysoPT, definitely provide a molecular basis for understanding of the mechanisms of activation of degranulation of MCs.

Experimental Section

Synthesis of LysoPS Analogues. General Methods. All reagents were purchased from Sigma-Aldrich Chemical Co., Tokyo Kasei Kogyo Co., Wako Pure Chemical Industries, and Kanto Kagaku Co., Inc. LysoPS (porcine brain) for bioassay was purchased from Avanti Polar Lipids. Silica gel for column chromatography was purchased from Kanto Kagaku Co., Inc. ^{1}H and ^{13}C ^{31}P NMR spectra were recorded on a Bruker Avance 400 spectrometer. Chemical shifts of ^{1}H- and ^{13}C-NMR spectra were shown in terms of parts per million (ppm), relative to those of chloroform (7.24 ppm for ^{1}H NMR spectra, and 77.00 ppm for ^{13}C NMR spectra). Chemical shifts of ^{31}P signals were reported in terms of parts per million (ppm), relative to that of phosphoric acid in water (85% w/w, as 0.00 ppm). Mass spectral data was recorded on a Bruker microTOF-05 (ESI-TOF) in the positive and negative ion detection modes.

General Procedure for Synthesis of Monodiisopropylamino Phosphoramidite (General Procedure A). Bis(diisopropylamino)-phosphite **27** (1.0 equiv) was dissolved in CH_2Cl_2. To this solution, the corresponding alcohol (1 equiv) was added. The resultant solution was dried by coevaporation with toluene. Under argon atmosphere, to a solution of the resultant mixture in CH_2Cl_2, a solution of 1*H*-tetrazole (1 equiv) in THF was added at room temperature. In a few minutes, white solids were precipitated. The whole was stirred for 2 h at room temperature, and the reaction was quenched with the addition of saturated aqueous $NaHCO_3$, and the whole was diluted with EtOAc. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine and was dried over Na_2SO_4. The solvent was evaporated, and the resultant residue was chromatographed to yield a pure product. In the purification with column chromatography, Et_3N deactivated silica gel was used, that is, eluents containing 3% (v/v) Et_3N.

General Procedure for Synthesis of Phosphate Triester Using Phosphoramidite Method and Oxidation (General Procedure B). Phosphoramidite (e.g., **28**) and an alcohol were dissolved in CH_2Cl_2. The resultant solution was dried by coevaporation with toluene. Under argon atmosphere, to a solution of the resultant mixture in CH_2Cl_2, a solution of 1*H*-tetrazole in THF was added at room temperature. In a few minutes, white solids were precipitated. The reaction mixture was stirred for 2 h at rt, and then a solution of *t*-butyl hydroperoxide (TBHP) in decane was added, and the whole was stirred for 1 h at rt. The obtained organic layer was washed with saturated aqueous $NaHCO_3$ and

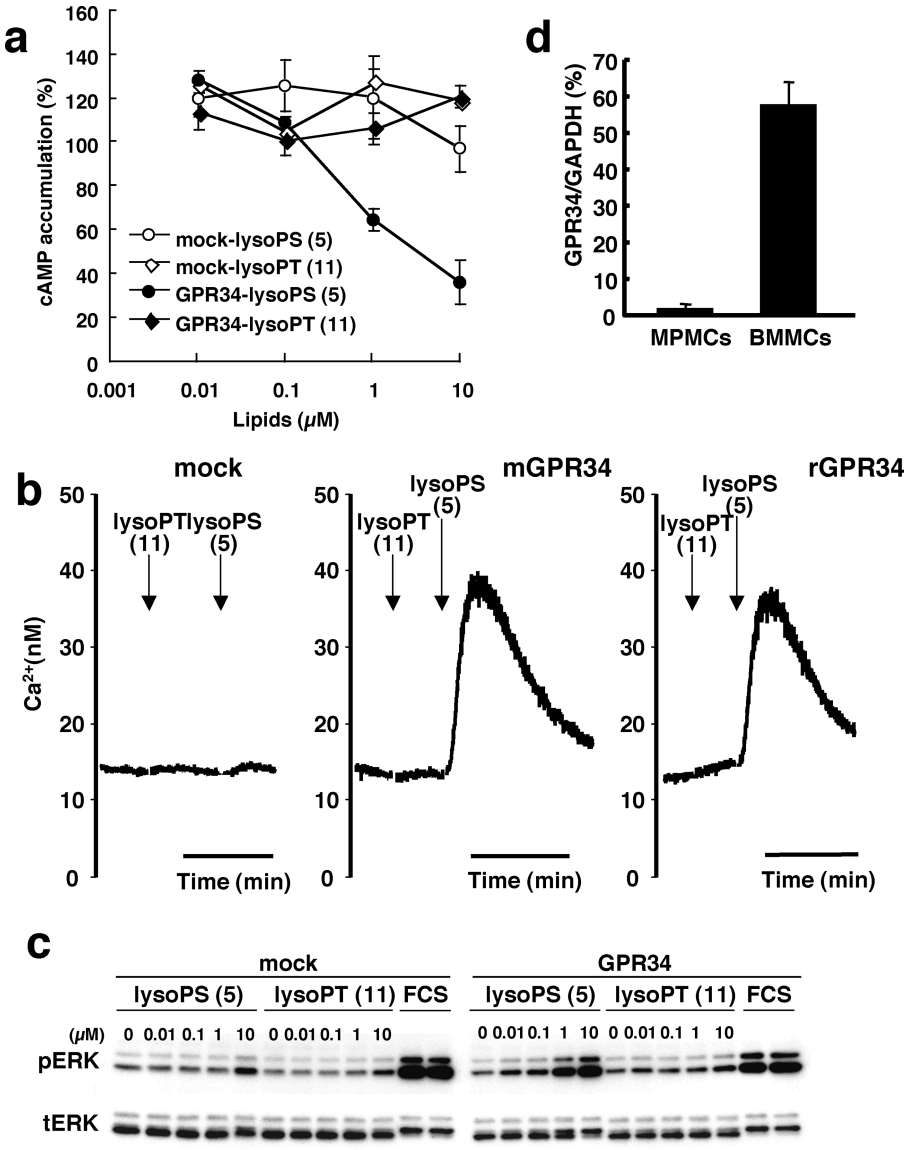


Figure 5. LysoPT does not activate GPR34. (a) Effect of lysoPT (**11**) on cAMP accumulation in forskolin-stimulated CHO-K1 cells stably expressing mouse GPR34 (CHO-GPR34). The cells were incubated with various concentrations of lysoPS (**5**) or lysoPT (**11**), and the forskolin-induced cAMP accumulation was measured by EIA assay. The cAMP level in the absence of ligand is defined as 100%. (b) Effect of lysoPT (**11**) on intracellular calcium mobilization ($[Ca^{2+}]_i$) in CHO-GPR34 cells. Fura-2-loaded CHO-GPR34 cells were stimulated with 2 μM lysoPS (**5**) or lysoPT (**11**), and $[Ca^{2+}]_i$ was determined fluorometrically using CAF-110. (c) Effect of lysoPT (**11**) on ERK activation in CHO-GPR34 cells. Serum-starved CHO-GPR34 cells were stimulated with either lysoPS (**5**) or lysoPT (**11**), and phosphorylated (pERK) and total ERK (tERK) were detected by Western blot analysis. Results from mock-transfected CHO cells are shown as a negative control. (d) GPR34 mRNA expression in mast cells. Total RNAs were prepared from mouse peritoneal mast cells (MPMCs) or mouse bone marrow-derived mast cells (BMMCs), and the amount of GPR34 RNA were analyzed by quantitative real-time PCR. The copy numbers for GPR34 were normalized by those of GAPDH.

brine and was dried over Na_2SO_4. The solvent was evaporated, and the resultant residue was chromatographed to yield a pure product.

General Procedure for Synthesis of Fatty Acid Ester from Fatty Acid Chloride (General Procedure C). An alcohol and a base (e.g., pyridine) were dissolved in CH_2Cl_2. To this solution, a solution of an acid chloride in CH_2Cl_2 was added dropwise at 0 °C, and the reaction mixture was stirred for 24 h. After quenching of the reaction with 3 M aqueous HCl, the obtained organic layer was washed with 3 M aqueous HCl and brine, and the whole was dried over Na_2SO_4. The solvent was evaporated, and the resultant residue was chromatographed to yield a pure product.

General Procedure for Synthesis of Fatty Acid Ester from Fatty Acid using EDCI and DMAP (General Procedure D). An alcohol, carboxylic acid, and *N*,*N*-dimethylaminopyridine (DMAP) were dissolved in CH_2Cl_2. To the solution was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) at 0 °C and then stirred for 24 h at room temperature. After quenching of the reaction with 3 M aqueous HCl, the organic layer was washed with 3 M aqueous HCl and brine, and the whole was dried over Na_2SO_4. The solvent was evaporated, and the resultant residue was chromatographed to yield a pure product.

General Procedure for Synthesis of *N*-benzyloxycarbonyl (Z) Amino Acid (General Procedure E). An amino acid was dissolved in a mixture of saturated $NaHCO_3$ and THF, and the whole was cooled to 0 °C. To this solution, a solution of benzyl chloroformate in THF was added at 0 °C and the whole was stirred for 1 h at 0 °C. After quenching the reaction with 3 M aqueous HCl

at 0 °C, the organic layer was washed with 3 M aqueous HCl and brine and dried over Na_2SO_4. The solvent was evaporated, and the resultant residue was chromatographed to yield a pure product.

General Procedure for Synthesis of *N*-Z Amino Acid Benzyl Ester (General Procedure F). A Z-amino acid, tetrabutylammonium iodide (TBAI), and Et_3N were dissolved in THF. To this solution was added a solution of benzyl bromide at room temperature. The reaction mixture was stirred for 16 h. The whole was washed with 3 M aqueous HCl, saturated $NaHCO_3$, and brine, and the whole was dried over Na_2SO_4. The solvent was evaporated, and the resultant residue was chromatographed to yield a pure product.

General Procedure for 4-Methoxytrityl (MMTr) or 4,4-Dimethyltrityl (DMTr) Protection of Alcohol (General Procedure G). To a solution of an alcohol and TBAI in CH_2Cl_2, diisopropylethylamine (DIPEA) was added. To this solution 4-methoxytriphenylmethyl chloride (MMTrCl) (or 4,4′-dimethoxytriphenylmethyl chloride (DMTrCl)) was added at rt, and the whole was stirred for 2 h. After quenching the reaction with 3 M aqueous HCl, saturated $NaHCO_3$, and brine, and the whole was dried over Na_2SO_4. The solvent was evaporated, and the resultant residue was chromatographed to yield a pure product.

General Procedure for Benzyl Etherification of Alcohol (General Procedure H). Under argon atmosphere to a solution of an alcohol and TBAI in THF, NaH (55% in mineral oil) was added at 0 °C and the whole was stirred for 10 min. Then, benzyl bromide was added to the solution at 0 °C, and the whole was stirred for 1 h at room temperature. After quenching the reaction with ice water, the obtained organic layer was diluted with EtOAc, and the whole was washed with brine and dried over Na_2SO_4. The solvent was evaporated, and the resultant residue was chromatographed to yield a pure product.

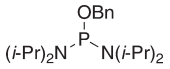
General Procedure for Methyl Etherification of Alcohol and *N*-Methylation of Amide (General Procedure I). Under argon atmosphere, to a solution of an alcohol in THF, NaH (55% in mineral oil) was added at 0 °C. The whole was stirred for 10 min, and then methyl iodide was added to this solution at 0 °C, and the whole was stirred for 2 h at rt. After quenching the reaction with ice water, the organic layer was diluted with EtOAc, and the whole was washed with brine and dried over Na_2SO_4. The solvent was evaporated, and the resultant residue was chromatographed to yield a pure product.

General Procedure for Deprotection of MMTr Group (General Procedure J). An MMTr-protected alcohol was dissolved in CH_2Cl_2-MeOH (1:3). To this solution a catalytic amount of *p*-toluenesulfonic acid (*p*-TSA) or camphorsulfonic acid (CSA) was added, and the whole was stirred for 30 min at room temperature. After quenching the reaction with Et_3N, the whole was washed with brine and dried over Na_2SO_4. The solvent was evaporated, and the resultant residue was chromatographed to yield a pure product.

General Procedure for Deprotection of 4-Methoxyphenyl (PMP) Group and Synthesis of Stearate (General Procedure K). A PMP-protected alcohol was dissolved in CH_3CN-H_2O, and the whole was cooled to 0 °C. To this solution, CAN was added and the whole was stirred for 20 min. After quenching the reaction with saturated aqueous $NaHCO_3$, the obtained organic layer was diluted with EtOAc, washed with saturated aqueous $NaHCO_3$, brine, and was dried over Na_2SO_4. The solvent was evaporated, and the resultant residue was chromatographed to yield a pure product. The product was dissolved in CH_2Cl_2, and DMAP was added and the whole was cooled to 0 °C. To this solution a solution of stearoyl chloride in CH_2Cl_2 was added dropwise at 0 °C, and the whole was stirred for 30 min. The solvent was evaporated, and the resultant residue was chromatographed to yield a pure product.

General Procedure for Simultaneous Deprotection of Benzyl and Benzyloxycarbonyl (Z) Protective Group (General Procedure L). A substrate was hydrogenated in MeOH-AcOH (4:1) over Pd−C (10%) for 1 day. The catalyst was filtered off through celite and the organic layer was evaporated and the resultant residue was chromatographed to yield a pure product.

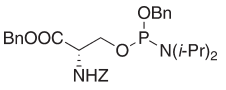
Synthesis of 2-Deoxy-lysoPS Analogues 1−4 (Scheme 1). Synthesis of Compound 1. Compound 27.



27

To a suspension of bis(diisopropylaminochlorophosphine) (15.0 g, 56.2 mmol) in dry Et_2O (300 mL), a solution of benzyl alcohol (6.21 mL, 60.0 mmol) and Et_3N (9.10 mL, 65.0 mmol) in Et_2O was added dropwise at 0 °C. The reaction mixture was stirred for 2 h at 0 °C and then the formed white solids were removed, the solvent of the filtrate was evaporated, and the residue was chromatographed (Et_3N/hexane 3:97) to yield **27** (17.89 g, 94%, colorless oil). ^{1}H NMR (CD_2Cl_2): δ 7.31 (5H, m), 4,63 (2H, d, J = 3.2 Hz), 3.57 (4H, m), 1.18 (24H, m). ^{13}C NMR ($CDCl_3$): δ 140.96, 140.86, 127.09, 127.07, 66.51, 66.28, 44.80, 44.67, 24.65, 24.57, 24.00, 23.94. HRMS (ESI, $[M + H]^+$): Calcd for $C_{19}H_{36}N_2OP$: 339.2565. Found: 339.2584.

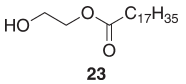
Compound 28.



28

Following the general procedure A, **27** (2.61 g, 7.70 mmol), *N*-Z-serine benzyl ester (2.31 g, 7.00 mmol), 1*H*-tetrazole (490.4 mg, 7.00 mmol), CH_2Cl_2 (40 mL), and THF (15 mL) were used. The crude mixture was chromatographed (EtOAc/hexane/Et_3N 20:80:3) to yield **28** (3.51 g, 6.19 mmol, 88%, colorless oil), a mixture of trans/cis isomers with respect to the benzyloxycarbonylamino group. ^{1}H NMR (CD_2Cl_2): δ 7.30 (15H, m), 5.86 ($^1/_2$H, d, J = 4.2 Hz), 5.65 ($^1/_2$H, d, J=4.2 Hz), 5.16 (2H, m), 5.08 (2H, m), 4.72−4.50 (3H, m), 4.15 (1H, m), 3.90 (1H, m), 3.58 (2H, m), 1.14 (12H, m). HRMS (ESI, $[M + Na]^+$): Calcd for $C_{31}H_{39}N_2NaO_6P$: 589.2443. Found: 589.2466.

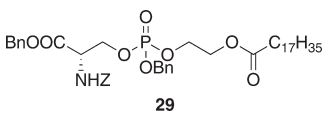
Compound 23.



23

Following the general procedure C, stearoyl chloride (1.68 g, 21.0 mmol), ethylene glycol (1.29 g, 5.55 mmol), CH_2Cl_2 (50 mL), and pyridine (1 mL) were used. The crude mixture was chromatographed (EtOAc/hexane 1:2 to 3:2) to yield **23** (1.27 g, 3.87 mmol, 70%, white solid); mp 56.0−56.5 °C. ^{1}H NMR ($CDCl_3$): δ 4.10 (2H, t, J=6.4 Hz), 3.68 (2H, t, J=6.4 Hz), 2.29 (2H, t, J=7.8 Hz), 1.55 (2H, m), 1.25 (28H, m), 0.88 (3H, t, J = 6.8 Hz). HRMS (ESI, $[M + Na]^+$): Calcd for $C_{20}H_{40}NaO_3$: 351.2875. Found: 351.2871.

Compound 29.

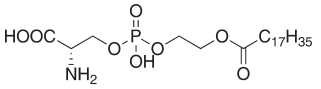


29

Following the general procedure B, **23** (82.4 mg, 0.251 mmol), **28** (170.0 mg, 0.298 mmol), 1*H*-tetrazole (52.6 mg, 0.750 mmol), TBHP (0.125 mL, 0.75 mmol), CH_2Cl_2 (1 mL), and THF (1.5 mL)

were used. The crude mixture was chromatographed (EtOAc/hexane 2:3) to yield **29** (140.8 mg, 0.168 mmol, 67%, colorless oil), a mixture of trans/cis isomers with respect to the benzyloxycarbonylamino group. ^{1}H NMR ($CDCl_3$): δ 7.31 (15H, m), 5.82 ($^1/_2$H, d, J = 4.2 Hz), 5.76 ($^1/_2$H, d, J = 4.2 Hz), 5.17 (2H, m), 5.09 (2H, m), 4.96 (2H, m), 4.58 (1H, m), 4.41 (1H, m), 4.27 (1H, m), 4.04 (2H, m), 2.25 (2H, t, J = 7 Hz), 1.55 (2H, m), 1.23(28H, m), 0.86 (3H, t, J = 6.8 Hz). ^{13}C NMR ($CDCl_3$): δ 173.43, 168.66, 155.78, 135.97, 135.26, 135.20, 134.86, 128.71, 128.58, 128.56, 128.48, 128.45, 128.41, 128.34, 128.26, 128.16, 128.11, 128.04, 127.94, 69.72, 69.67, 67.68, 67.31, 67.13, 65.74, 65.69, 62.49, 62.42, 54.41, 33.89, 31.85, 29.62, 29.58, 29.54, 29.40, 29.29, 29.20, 29.04, 24.70, 22.62, 12.06. ^{31}P NMR ($CDCl_3$): δ −0.67. HRMS (ESI, $[M + Na]^+$): Calcd for $C_{45}H_{64}NNaO_{10}P$: 832.4166. Found: 832.4184.

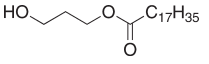
Compound 1.



1

Following the general procedure L, **29** (34.0 mg, 0.0406 mmol), Pd−C (3.0 mg), and MeOH-AcOH (5 mL, 4:1) were used. The crude mixture was chromatographed ($CHCl_3$/MeOH/AcOH 9:0:1 to 8:1:1), and the product was washed with methanol to yield **1** (15.4 mg, 0.0294 mmol, 72%, white powder). ^{1}H NMR ($CDCl_3$/CD_3CO_2D 4:1): δ 4.34 (2H, m), 4.15 (3H, m), 4.00 (2H, m), 2.25 (2H, m), 1.53 (4H, m), 1.17 (28H, m), 0.79 (3H, t, J = 6.6 Hz). HRMS (ESI, $[M - H]^-$): Calcd for $C_{23}H_{45}NO_8P$: 494.2883. Found: 494.2880. Anal. Calcd for $C_{23}H_{46}NO_8P + {}^1/_3CF_3CO_2H$: C, 53.27; H, 8.75; N, 2.62. Found: C, 53.22; H, 8.54; N, 2.55. mp 155.5−156.0 °C.

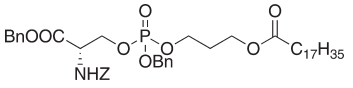
Synthesis of Compound 2 (2-Deoxy-lysoPS). Compound 24.



24

Following the general procedure C, stearoyl chloride (612.7 mg, 2.20 mmol), 1,3-propane diol (617.4 mg, 8.11 mmol), CH_2Cl_2 (3 mL), and pyridine (0.2 mL) were used. The crude mixture was chromatographed (EtOAc/hexane 1:3) to yield **24** (453.8 mg, 1.44 mmol, 66%, white solid). ^{1}H NMR ($CDCl_3$): δ 4.10 (2H, t, J = 6.4 Hz), 3.68 (2H, t, J = 6.4 Hz), 2.29 (2H, t, J = 7.8 Hz), 1.72 (2H, m), 1.60 (1H, m), 1.53 (2H, m), 1.25 (28H, m), 0.88 (3H, t, J = 6.8 Hz). HRMS (ESI, $[M + Na]^+$): Calcd for $C_{21}H_{42}NaO_3$: 365.3032. Found: 365.3038. mp 51.5−52.0 °C.

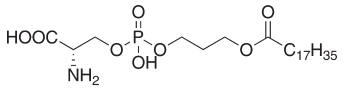
Compound 30.



30

Following the general procedure B, **24** (128.7 mg, 0.360 mmol), **28** (240.7 mg, 0.420 mmol), 1*H*-tetrazole (62.9 mg, 0.900 mmol), TBHP (0.2 mL, 1.20 mmol), CH_2Cl_2 (2 mL), and THF (1.8 mL) were used. The crude mixture was chromatographed (EtOAc/hexane 1:3 to 1:2) to yield **30** (240.3 mg, 0.292 mmol, 81%, colorless oil). A mixture of trans/cis isomers with respect to the benzyloxycarbonylamino group was present. ^{1}H NMR ($CDCl_3$): δ 7.26 (15H, m), 5.86 ($^1/_2$H, d, J = 4.2 Hz), 5.78 ($^1/_2$H, d, J = 4.2 Hz), 5.18 (2H, m), 5.11 (2H, m), 4.98 (2H, m), 4.60 (1H, m), 4.44 (1H, m), 4.28 (1H, m), 4.07 (2H, m), 3.98 (2H, m), 2.25 (2H, t, J = 7.0 Hz), 1.87 (2H, m), 1.55 (2H, m), 1.25(28H, m), 0.88 (3H, t, J = 6.4 Hz). ^{13}C NMR ($CDCl_3$): δ 173.68, 168.74, 155.81, 136.01, 135.44, 135.38, 134.90, 128.72, 128.62, 128.50, 128.29, 128.20, 128.09, 127.98, 77.21, 69.66, 69.61, 67.71, 67.17, 64.74, 60.00, 54.44, 34.13, 31.85, 29.6, 29.63, 29.59, 29.45, 29.3, 29.25, 29.13, 24.86, 22.66, 14.10. ^{31}P NMR ($CDCl_3$): δ −0.42, −0.44. HRMS (ESI, $[M + Na]^+$): Calcd for $C_{46}H_{66}NNaO_{10}P$: 846.4322. Found: 846.4351.

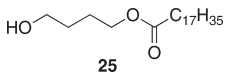
Compound 2 (2-Deoxy-lysoPS).



2

Following the general procedure L, **30** (50.0 mg, 0.0606 mmol), Pd−C (3.0 mg), and MeOH-AcOH (5 mL) were used. The crude mixture was chromatographed ($CHCl_3$/MeOH/AcOH 9:0:1 to 8:1:1), and the product was washed with MeOH to yield **2** (18.2 mg, 0.0357 mmol, 59%, white powder). ^{1}H NMR ($CDCl_3$/CD_3COOD 4:1): δ 4.43 (1H, m), 4.33 (1H, m), 4.23 (1H, m), 4.06 (2H, m), 3.90 (2H, m), 2.20 (2H, t, J = 6.4 Hz), 1.48(4H, m), 1.13 (28H, m), 0.74 (3H, t, J = 6.8 Hz). HRMS (ESI, $[M - H]^-$): Calcd for $C_{24}H_{47}NO_8P$: 508.3039. Found: 508.3014. Anal. Calcd for $C_{24}H_{48}NO_8P + {}^3/_5CF_3CO_2H$: C, 52.36; H, 8.47; N, 2.42. Found: C, 52.35; H, 8.44; N, 2.39. mp 150.5−151.0 °C.

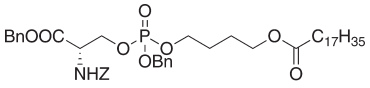
Synthesis of Compound 3. Compound 25.



25

Following the general procedure C, stearoyl chloride (3.02 g, 10.0 mmol), 1,4-butane diol (3.63 g, 40.0 mmol), CH_2Cl_2 (50 mL), and pyridine (2 mL) were used. The crude mixture was chromatographed (EtOAc/hexane 1:3) to yield **25** (2.45 g, 6.87 mmol, 69%, white solid). ^{1}H NMR ($CDCl_3$): δ 4.10 (2H, t, J = 6.4 Hz), 3.68 (2H, t, J = 6.4 Hz), 2.29 (2H, t, J = 7.8 Hz), 1.72 (2H, m), 1.48 (4H, m), 1.25 (28H, m), 0.88 (3H, t, J = 6.8 Hz). HRMS (ESI, $[M + Na]^+$): Calcd for $C_{22}H_{44}NaO_3$: 379.3188. Found: 379.3183. mp 40.5−41.0 °C.

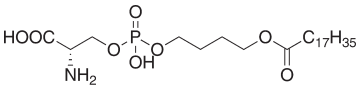
Compound 31.



31

Following the general procedure B, **25** (177.9 mg, 0.500 mmol), **28** (304.4 mg, 0.550 mmol), 1*H*-tetrazole (62.9 mg, 0.900 mmol), TBHP (0.2 mL, 1.20 mmol), CH_2Cl_2 (2 mL), and THF (1.8 mL) were used. The crude mixture was chromatographed (EtOAc/hexane 1:3 to1:2) to yield **31** (339.4 mg, 0.405 mmol, 81%, colorless oil). ^{1}H NMR ($CDCl_3$): δ 7.32 (15H, m), 5.85 ($^1/_2$H, d, J = 4.2 Hz), 5.78 ($^1/_2$H, d, J = 4.2 Hz), 5.18 (2H, m), 5.11 (2H, m), 4.98 (2H, m), 4.58 (1H, m), 4.41 (1H, m), 4.28 (1H, m), 4.01 (2H, m), 3.92 (2H, m), 2.26 (2H, t, J = 7.5 Hz), 1.61 (4H, m), 1.56 (2H, m), 1.25 (28H, m), 0.88 (3H, t, J = 6.4 Hz). ^{13}C NMR ($CDCl_3$): δ 173.81, 168.74, 155.79, 136.00, 135.47, 135.44, 134.89, 128.68, 128.59, 128.48, 128.25, 128.18, 128.07, 127.97, 77.20, 69.63, 69.57, 67.69, 67.15, 63.38, 54.44, 34.22, 31.87, 29.65, 29.61, 29.56, 29.43, 29.31, 29.23, 29.12, 24.90, 24.73, 22.64, 14.08. ^{31}P NMR ($CDCl_3$): δ −0.40, −0.46. HRMS (ESI, $[M + Na]^+$): Calcd for $C_{47}H_{68}NNaO_{10}P$: 860.4479. Found: 860.4467.

Compound 3.

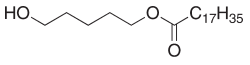


3

Following the general procedure L, **31** (61.6 mg, 0.0736 mmol), Pd−C (3.0 mg), and MeOH-AcOH (5 mL) were used. The crude

mixture was chromatographed ($CHCl_3$/MeOH/AcOH 9:0:1 to 8:1:1), and the product was washed with MeOH to yield **3** (25.2 mg, 0.0481 mmol, 65%, white powder). ^{1}H NMR ($CDCl_3$/CD_3CO_2D 4:1): δ 4.39 (1H, m), 4.29 (1H, m), 4.23 (1H, m), 3.94 (2H, m), 3.81 (2H, m), 2.16 (2H, t, J = 7.6 Hz), 1.55 (4H, m), 1.45 (2H, m), 1.13 (28H, m), 0.73 (3H, t, J = 8.0 Hz). HRMS (ESI, $[M - H]^-$) Calcd for $C_{25}H_{49}NO_8P$: 522.3196. Found: 522.3192. Anal. Calcd for $C_{25}H_{50}NO_8P + {}^1/_3CF_3CO_2H$: C, 55.47; H, 9.17; N, 2.54. Found: C, 55.76; H, 9.03; N, 2.56. mp 143.5−144.0 °C.

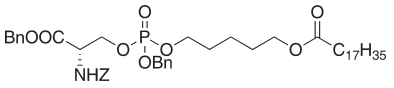
Synthesis of Compound 4. Compound 26.



26

Following the general procedure C, stearoyl chloride (3.05 g, 10.1 mmol), 1,4-pentane diol (4.23 g, 40.0 mmol), CH_2Cl_2 (50 mL), and pyridine (2 mL) were used. The crude mixture was chromatographed (EtOAc/hexane 1:3) to yield **26** (2.84 g, 1.44 mmol, 76%, white solid). ^{1}H NMR ($CDCl_3$): δ 4.08 (2H, t, J = 6.6 Hz), 3.66 (2H, t, J = 6.4 Hz), 2.29 (2H, t, J = 7.6 Hz), 1.64 (3H, m), 1.48 (2H, m), 1.43 (4H, m), 1.25 (28H, m), 0.88 (3H, t, J = 6.8 Hz). HRMS (ESI, $[M + Na]^+$): Calcd for $C_{23}H_{46}NaO_3$ 393.3345. Found: 393.3339. mp 39.5−40.0 °C.

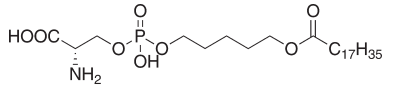
Compound 32.



32

Following the general procedure B, **26** (238.0 mg, 0.360 mmol), **28** (133.3 mg, 0.360 mmol), 1*H*-tetrazole (52.6 mg, 0.750 mmol), TBHP (0.1 mL, 0.600 mmol), CH_2Cl_2 (2 mL), and THF (1.5 mL) were used. The crude mixture was chromatographed (EtOAc/hexane 1:3 to 1:2) to yield **32** (254.0 mg, 0.298 mmol, 83%, colorless oil). ^{1}H NMR ($CDCl_3$): δ 7.27 (15H, m), 5.85 ($^1/_2$H, d, J = 4.2 Hz), 5.78 ($^1/_2$H, d, J = 4.2 Hz), 5.18 (2H, m), 5.10 (2H, m), 4.98 (2H, m), 4.58 (1H, m), 4.41 (1H, m), 4.28 (1H, m), 4.01 (2H, m), 3.90 (2H, m), 2.27 (2H, t, J = 7.5 Hz), 1.56 (8H, m), 1.25(28H, m), 0.87 (3H, t, J = 6.8 Hz). ^{13}C NMR ($CDCl_3$): δ 173.90, 168.77, 155.79, 136.01, 135.54, 135.48, 134.91, 128.67, 128.60, 128.50, 128.24, 128.20, 128.09, 127.96, 69.57, 69.51, 67.95, 67.70, 67.10, 63.85, 54.53, 34.18, 31.89, 29.66, 29.63, 29.58, 29.45, 29.33, 29.25, 29.14, 28.07, 24.94, 22.66, 21.82, 14.08. ^{31}P NMR ($CDCl_3$): δ −0.29, −0.34. HRMS (ESI, $[M + Na]^+$): Calcd for $C_{48}H_{70}NNaO_{11}P$: 874.4636. Found: 874.4629.

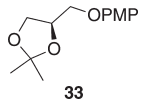
Compound 4.



4

Following the general procedure L, **32** (62.8 mg, 0.0737 mmol), Pd−C (3.0 mg), and MeOH-AcOH (5 mL) were used. The crude mixture was chromatographed ($CHCl_3$/MeOH/AcOH 9:0:1 to 8:1:1), and the product was washed with MeOH to yield **4** (27.1 mg, 0.0504 mmol, 68%, white powder). ^{1}H NMR ($CDCl_3$/CD_3CO_2D 4:1): δ 4.36 (1H, m), 4.23 (1H, m), 3.89 (2H, m), 3.74 (2H, m), 2.13 (2H, t, J = 7.4 Hz), 1.55 (6H, m), 1.45 (3H, m), 1.08 (28H, m), 0.68 (3H, t, J = 8.0 Hz). HRMS (ESI, $[M - H]^-$): Calcd for $C_{26}H_{51}NO_8P$: 536.3352. Found: 536.3341. Anal. Calcd for $C_{26}H_{52}NO_8P + {}^1/_3CF_3CO_2H$: C, 52.71; H, 8.46; N, 2.23. Found: C, 52.38; H, 8.87; N, 2.40. mp 146.5−147.0 °C.

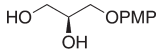
Synthesis of Lyso-PS Derivatives 5−8 (Scheme 2). Synthesis of Compound 5. Compound 33.



33

(*R*)-(−)-2,2-Dimethyl-1,3-dioxolane-4-methanol (5.00 g, 37.8 mmol), triphenylphosphine (12.90 g, 49.2 mmol), and *p*-methoxyphenol (14.09 g, 113.5 mmol) was dissolved in toluene. To the solution DEAD (2.2 M in toluene, 22.4 mL, 49.3 mmol) was added and warmed to 70 °C and stirred for 2 h. The reaction mixture was evaporated and chromatographed (Et_2O/hexane 1:15) to yield **33** (8.487 g, 35.62 mmol, 94%, colorless oil). ^{1}H NMR ($CDCl_3$): δ 6.82 (4H, m), 4.44 (1H, m), 4.13 (1H, m), 3.99 (1H, m), 3.88 (1H, m), 3.86 (1H, m), 3.74 (3H, s), 1.43 (3H, s), 1.38 (3H, s). ^{13}C NMR ($CDCl_3$): δ d 153.96, 152.62, 121.78, 115.37, 114.48, 114.28, 109.53, 73.99, 69.40, 66.73, 55.53, 55.42, 26.67, 25.25. HRMS (ESI, $[M + Na]^+$): Calcd for $C_{13}H_{18}NaO_4$: 261.1103. Found: 261.1109.

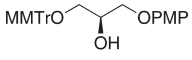
Compound 35.



35

To a solution of **33** (1.00 g, 4.20 mmol) in MeOH (150 mL), Amberlyst-15 (655.2 mg) was added, and the whole was stirred for 1 d at room temperature. Amberlyst-15 was filtered off, the solvent of the filtrate was evaporated, and the residue was chromatographed (EtOAc/hexane 2:1 to 4:1) to yield **35** (1.13 g, 3.80 mmol, 90%, colorless oil). ^{1}H NMR ($CDCl_3$): δ 6.82 (4H, m), 4.07 (1H, m), 3.99 (2H, m), 3.81 (1H, m), 3.75 (3H, s), 3.72 (1H, m), 2.05 (2H, bs). ^{13}C NMR ($CDCl_3$): δ 154.00, 152.48, 115.44, 114.57, 70.55, 69.68, 63.63, 55.58. HRMS (ESI, $[M + Na]^+$): Calcd for $C_{10}H_{14}NaO_4$: 221.0790. Found: 221.0773. mp 74.5−75.0 °C.

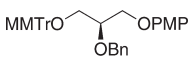
Compound 37.



37

Following the general procedure G, **35** (695.6 mg, 3.51 mmol), TBAI (394.2 mg, 1.07 mmol), DIPEA (1.36 g, 1.83 mL, 10.5 mmol), 4-methoxy tritylchloride (1.18 g, 3.83 mmol), and CH_2Cl_2 (30 mL) were used. The crude mixture was chromatographed (EtOAc/hexane 1:4 to 1:2) to yield **37** (1.57 g, 3.20 mmol, 91%, yellow oil). ^{1}H NMR ($CDCl_3$): δ 7.41 (6H, m), 7.30−7.18 (8H, m), 6.80 (4H, m), 4.09 (1H, m), 3.99 (2H, m), 3.77 (3H, s), 3.75 (3H, s), 3.30 (2H, m). ^{13}C NMR ($CDCl_3$): δ 158.50, 153.92, 152.60, 147.07, 144.18, 144.16, 135.28, 130.28, 129.14, 128.28, 127.78, 127.03, 126.88, 115.45, 114.58, 114.53, 113.07, 86.41, 70.33, 69.79, 69.72, 69.47, 64.10, 63.54, 55.60, 55.09. HRMS (ESI, $[M + Na]^+$): Calcd for $C_{30}H_{30}NaO_5$: 493.1991. Found: 493.2000.

Compound 39.

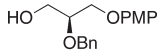


39

Following the general procedure H, **37** (1.39 g, 2.96 mmol), TBAI (328.7 mg, 0.89 mmol), NaH (323.0 mg, 7.40 mmol), benzyl bromide (760.4 mg, 4.45 mmol), and THF (30 mL) were used. The crude mixture was chromatographed (EtOAc/hexane

1:15 to 1:12) to yield **39** (1.48 g, 2.64 mmol, 89%, yellow oil). ^{1}H NMR ($CDCl_3$): δ 7.42 (4H, m), 7.35−7.18 (11H, m), 6.78 (8H, m), 4.66 (2H, m), 4.05 (2H, m), 3.88 (1H, m), 3.76 (3H, s), 3.74 (3H, s), 3.31 (2H, m). ^{13}C NMR ($CDCl_3$): δ 158.41, 153.74, 152.81, 144.36, 144.34, 138.38, 135.45, 130.28, 128.32, 128.21, 127.69, 127.46, 126.74, 115.43, 114.44, 112.96, 86.33, 72.18, 68.80, 63.02, 55.51, 55.00. HRMS (ESI, $[M + Na]^+$): Calcd for $C_{37}H_{36}NaO_5$: 583.2460. Found: 583.2476.

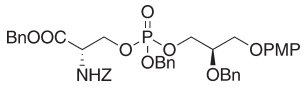
Compound 43.



43

Following the general procedure J, **39** (1.45 g, 2.64 mmol), CSA (183.6 mg, 0.790 mmol), and CH_2Cl_2-MeOH (20 mL) were used. The crude mixture was chromatographed (EtOAc/hexane 1:3) to yield **43** (673.7 mg, 2.22 mmol, 84%, white solid). ^{1}H NMR ($CDCl_3$): δ 7.34 (5H, m), 6.81 (4H, m), 4.72 (2H, m), 4.03 (2H, m), 3.87 (1H, m), 3.84 (1H, m), 3.75 (3H, s), 3.73 (1H, m). ^{13}C NMR ($CDCl_3$): δ 153.92, 152.45, 115.39, 114.52, 70.55, 69.58, 63.58, 55.51. HRMS (ESI, $[M + Na]^+$): Calcd for $C_{17}H_{20}NaO_4$: 311.1259. Found: 311.1252. mp 44.5−45.0 °C.

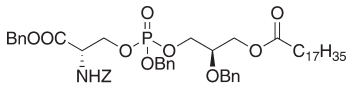
Compound 47.



47

Following the general procedure B, **43** (151.7 mg, 0.500 mmol), **28** (337.2 mg, 0.610 mmol), 1*H*-tetrazole (105.7 mg, 1.51 mmol), TBHP (0.42 mL, 2.52 mmol), CH_2Cl_2 (2 mL), and THF (3 mL) were used. The crude mixture was chromatographed (EtOAc/hexane 3:7) to yield **47** (291.1 mg, 0.378 mmol, 76%, colorless oil). ^{1}H NMR ($CDCl_3$): δ 7.26−7.30 (20H, m), 6.76 (4H, m), 5.88 (1H, m), 5.14 (2H, m), 5.06 (2H, m), 4.93 (2H, m), 4.63 (2H, m), 4.57 (1H, m), 4.38 (1H, m), 4.23 (1H, m), 4.21(1H, m), 4.09 (1H, m), 3.90 (2H, m), 3.84 (1H, m), 3.73 (3H, s). HRMS (ESI, $[M + Na]^+$): Calcd for $C_{42}H_{44}NNaO_{11}P$: 792.2550. Found: 792.2550.

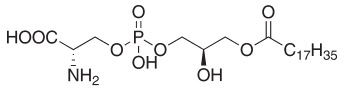
Compound 51.



51

Following the general procedure K, **47** (154.0 mg, 0.200 mmol), CAN (266.7 mg, 0.487 mmol), and CH_3CN-H_2O (2 mL) were used. The crude mixture was chromatographed (EtOAc/hexane 1:2 to 3:2) to yield the alcohol (104.2 mg, 0.153 mmol, 77%, brown oil). The resultant alcohol (104.2 mg, 0.153 mmol) was acylated with stearoyl chloride (55.6 mg, 0.183 mmol) in CH_2Cl_2 (1.5 mL) in the presence of DMAP (56.0 mg, 0.458 mmol). The crude mixture was chromatographed (EtOAc/hexane 1:3 to 2:3) to yield **51** (112.2 mg, 0.121 mmol, 79%, colorless oil). ^{1}H NMR ($CDCl_3$): δ 7.27 (20H, m), 5.86 (1H, m), 5.13 (2H, m), 5.08 (2H, m), 4.95 (2H, m), 4.55 (3H, m), 4.40 (1H, m), 4.26 (1H, m), 4.15 (2H, m), 4.05 (1H, m), 3.96 (1H, m), 3.70 (1H, m), 2.24 (2H, t, J = 7.6 Hz), 1.55 (2H, m), 1.23 (28H, m), 0.86 (3H, t, J = 6.8 Hz). ^{13}C NMR ($CDCl_3$): δ 173.35, 168.69, 155.79, 137.52, 137.46, 136.01, 135.33, 134.89, 128.65, 128.57, 128.46, 128.38, 128.35, 128.21, 128.14, 128.05, 127.93, 127.85, 127.80, 74.93, 74.86, 74.81, 72.04, 72.01, 69.66, 69.60, 67.64, 67.10, 66.53, 66.46, 62.15, 54.47, 54.41, 34.01, 31.86, 29.64, 29.60, 29.57, 29.42, 29.30, 29.22, 29.08, 24.79, 22.63, 14.07. ^{31}P NMR ($CDCl_3$): δ −0.22, −0.24. HRMS (ESI, $[M + Na]^+$): Calcd for $C_{53}H_{72}NNaO_{11}P$: 952.4741. Found: 952.4744.

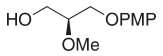
Compound 5 (LysoPS).



5 (2*R*-OH)

Following the general procedure L, using **51** (30.0 mg, 0.0323 mmol), Pd−C (3.0 mg), and MeOH-AcOH (5 mL). The crude mixture was chromatographed ($CHCl_3$/MeOH/AcOH 9:0:1 to 8:1:1 to 7:2:1), and the product was washed with MeOH to yield **5** (9.2 mg, 0.0175 mmol, 58%, white solid). ^{1}H NMR ($CDCl_3$/CD_3CO_2D 4:1): δ 4.85−4.34 (3H, m), 4.32−3.88 (5H, m), 2.23 (2H, m), 1.55(2H, m), 1.08 (28H, m), 0.76 (3H, t, J = 7.0 Hz). HRMS (ESI, $[M - H]^-$) Calcd for $C_{24}H_{47}NO_9P$: 524.2988. Found: 524.2964. Anal. Calcd for $C_{24}H_{48}NO_9P + {}^4/_5CF_3CO_2H$: C, 49.85; H, 7.97; N, 2.27. Found: C, 49.64; H, 8.04; N, 2.59. mp 160.0−160.5 °C.

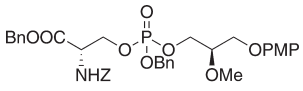
Synthesis of Compound 6. Compound 44.



44

Following the general procedure J, **40** (1.30 g, 2.68 mmol), *p*-toluenesulfonic acid (51.0 mg, 0.268 mmol), and CH_2Cl_2-MeOH (30 mL) were used. The crude mixture was chromatographed (EtOAc/hexane 1:2 to 1:1) to yield **44** (475.4 mg, 2.24 mmol, 84%, white solid). ^{1}H NMR ($CDCl_3$): δ 6.82 (4H, m), 4.03(2H, m), 3.83 (1H, m), 3.75 (3H, s), 3.71 (1H, m), 3.63 (1H, m), 3.51 (3H, m), 1.76 (1H, bs). HRMS (ESI, $[M + Na]^+$): Calcd for $C_{11}H_{16}NaO_4$: 235.0946. Found: 235.0951. mp 48.0−48.5 °C.

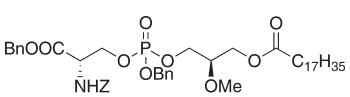
Compound 48.



48

Following the general procedure B, **44** (106.5 mg, 0.502 mmol), **28** (303.2 mg, 0.535 mmol), 1*H*-tetrazole (96.3 mg, 1.38 mmol), TBHP (0.42 mL, 2.52 mmol), CH_2Cl_2 (2 mL), and THF (3 mL) were used. The crude mixture was chromatographed (EtOAc/hexane 3:7) to yield **48** (267.5 mg, 0.386 mmol, 77%, colorless oil). ^{1}H NMR ($CDCl_3$): δ 7.28−7.31 (15H, m), 6.76 (4H, m), 5.92 (1H, m), 5.15 (2H, m), 5.08 (2H, m), 4.97 (2H, m), 4.56 (1H, m), 4.41 (1H, m), 4.29 (1H, m), 4.19 (1H, m), 4.09 (1H, m), 3.88 (2H, m), 3.73 (3H, s), 3.62 (1H, m), 3.40 (3H, m). HRMS (ESI, $[M + Na]^+$): Calcd for $C_{36}H_{40}NnaO_{11}P$: 716.2237. Found: 716.2237.

Compound 52.

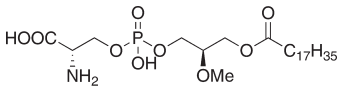


52

Following the general procedure K, **48** (216.5 mg, 0.312 mmol), CAN (427.8 mg, 0.780 mmol), and CH_3CN-H_2O (3 mL) were used. The crude mixture was chromatographed (EtOAc/hexane 1:1 to 4:1) to yield the intermediate alcohol (142.8 mg, 0.243 mmol, 77%, brown oil). The obtained alcohol (140.3 mg, 0.239 mmol)

was acylated with stearoyl chloride (79.6 mg, 0.263 mmol) in CH_2Cl_2 (2.5 mL) in the presence of DMAP (87.5 mg, 0.716 mmol). The crude mixture was chromatographed (EtOAc/hexane 1:3 to 2:3) to yield **52** (155.9 mg, 0.183 mmol, 76%, colorless oil). ^{1}H NMR ($CDCl_3$): δ 7.30 (15H, m), 5.90 (1H, m), 5.16 (2H, m), 5.09 (2H, m), 4.97 (2H, m), 4.58 (1H, m), 4.43 (1H, m), 4.30 (1H, m), 4.11 (1H, m), 4.05−3.90 (3H, m), 3.47 (1H, m), 3.35 (3H, s), 2.26 (2H, t, J=7.6 Hz), 1.55 (2H, m), 1.23 (28H, m), 0.86 (3H, t, J= 7.0 Hz). ^{13}C NMR ($CDCl_3$): δ 173.44, 168.75, 155.85, 136.05, 135.40, 134.93, 128.73, 128,63, 128.62, 128.50, 128.27, 128.19, 128.08, 128.01, 127.99, 69.70, 67.70, 67.45, 67.15, 66.36, 66.30, 65.84, 61.81, 58.01, 58.79, 54.52, 54.45, 34.04, 31.90, 29.68, 29.64, 29.60, 29.46, 29.34, 29.25, 29.10, 24.83, 22,67, 14.11. ^{31}P NMR ($CDCl_3$): δ −0.34, −0.40. HRMS (ESI, $[M + Na]^+$) Calcd for $C_{47}H_{68}NNaO_{11}P$: 876.4428. Found: 876.4391.

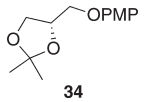
Compound 6.



6 (2*R*-MeO)

Following the general procedure L, **52** (27.9 mg, 0.0326 mmol), Pd−C (3.0 mg), and MeOH-AcOH (5 mL) were used. The crude mixture was chromatographed ($CHCl_3$/MeOH/AcOH 9:0:1 to 8:1:1 to7:2:1) and the product was washed with MeOH to yield **6** (8.0 mg, 0.0166 mmol, 51%, white powder). ^{1}H NMR ($CDCl_3$/CD_3CO_2D 4:1): δ 4.85−4.34 (3H, m), 4.32−3.88 (5H, m), 3.51 (1H, m), 3.35 (3H, s) 2.23 (2H, m), 1.55 (2H, m), 1.08 (28H, m), 0.76 (3H, t, J = 7.0 Hz). HRMS (ESI, $[M - H]^-$) Calcd for $C_{25}H_{49}NO_9P$: 538.3145. Found: 538.3131. Anal. Calcd for $C_{25}H_{50}NO_9P + CF_3CO_2H$: C, 49.61; H, 7.86; N, 2.14. Found: C, 49.56; H, 8.07; N, 2.22. mp 175.5−176.0 °C.

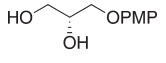
Synthesis of Compound 7. Compound 34.



34

To a solution of (*S*)-(+)-2,2-dimethyl-1,3-dioxolane-4-methanol (1.33 g, 1.01 mmol), triphenylphosphine (3.13 g, 12.0 mmol), and *p*-methoxyphenol (1.37 g, 1.10 mmol) in toluene (60 mL), diethyl azodicarboxylate (DEAD) (2.2 M in toluene, 5.5 mL, 12.1 mmol) was added. The whole was warmed to 70 °C, and stirred for 2 h. The solvent was evaporated and the residue was chromatographed (Et_2O/hexane 1:15) to yield **34** (1.577 g, 6.63 mmol, 66%, colorless oil). ^{1}H NMR ($CDCl_3$): δ 6.82 (4H, m), 4.43 (1H, m), 4.13 (1H, m), 4.02 (1H, m), 3.88 (1H, m), 3.85 (1H, m), 3.74 (3H, s), 1.43 (3H, s), 1.38 (3H, s). HRMS (ESI, $[M + Na]^+$): Calcd for $C_{13}H_{18}NaO_4$: 261.1103. Found: 261.1101.

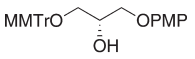
Compound 36.



36

To a solution of **34** (1.45 g, 6.09 mmol) in MeOH (160 mL), Amberlyst-15 (792.7 mg) was added and the whole was stirred for 1 d at room temperature. Amberlyst-15 was removed by filtration and the solvent was evaporated. The residue was chromatographed (EtOAc/hexane 2:1 to 4:1) to yield **36** (923.2 mg, 4.73 mmol, 78%, colorless oil). ^{1}H NMR ($CDCl_3$): δ 6.82 (4H, m), 4.07 (1H, m), 3.98 (2H, m), 3.81 (1H, m), 3.75 (3H, s), 3.72 (1H, m), 2.23 (2H, bs). HRMS (ESI, $[M + Na]^+$): Calcd for $C_{10}H_{14}NaO_4$: 221.0790, Found: 221.0780. mp 71.5−72.0 °C.

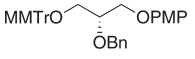
Compound 38.



38

Following the general procedure G, **36** (807.7 mg, 4.08 mmol), TBAI (451.7 mg, 1.22 mmol), DIPEA (1.58 g, 2.13 mL, 12.23 mmol), 4-methoxytritylchloride (1.384 g, 4.48 mmol), and CH_2Cl_2 (20 mL) were used. The crude mixture was chromatographed (EtOAc/hexane 1:4 to 1:2) to yield **38** (1.68 g, 3.59 mmol, 88%, yellow oil). ^{1}H NMR ($CDCl_3$): δ 7.41 (6H, m), 7.30−7.19 (8H, m), 6.80 (4H, m), 4.10 (1H, m), 3.99 (2H, m), 3.77 (3H, s), 3.75 (3H, s), 3.30 (2H, m). HRMS (ESI, $[M + Na]^+$): Calcd for $C_{30}H_{30}NaO_5$: 493.1991. Found: 493.1990.

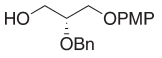
Compound 41.



41

Following the general procedure H, **38** (1.688 g, 3.59 mmol), TBAI (397.4 mg, 1.08 mmol), NaH (391.2 mg, 8.97 mmol), benzyl bromide (920.1 mg, 0.64 mL, 5.38 mmol), and THF (20 mL) were used. The crude mixture was chromatographed (EtOAc/hexane 1:15 to 1:12) to yield **41** (1.772 g, 3.16 mmol, 88%, yellow oil). ^{1}H NMR ($CDCl_3$): δ 7.44 (4H, m), 7.32−7.19 (11H, m), 6.79 (8H, m), 4.66 (2H, m), 4.06 (2H, m), 3.88 (1H, m), 3.76 (3H, s), 3.74 (3H, s), 3.31 (2H, m). HRMS (ESI, $[M + Na]^+$): Calcd for $C_{37}H_{36}NaO_5$: 583.2460. Found: 583.2461.

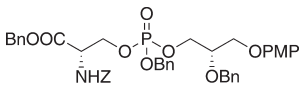
Compound 45.



45

Following the general procedure J, **41** (1.66 g, 2.96 mmol), *p*-toluenesulfonic acid (66.4 mg, 0.347 mmol), and CH_2Cl_2-MeOH (40 mL) were used. The crude mixture was chromatographed (EtOAc/hexane 3:7) to yield **45** (672.9 mg, 1.99 mmol, 67%, white solid). ^{1}H NMR ($CDCl_3$): δ 7.35−7.28 (5H, m), 6.81 (4H, m), 4.75(1H, m), 4.66 (1H, m), 4.03 (2H, m), 3.85 (1H, m), 3.82 (1H, m), 3.75 (3H, s), 3.73 (1H, m), 1.75 (1H, bs). HRMS (ESI, $[M + Na]^+$): Calcd for $C_{17}H_{20}NaO_4$: 311.1259. Found: 311.1259. mp 44.5−45.0 °C.

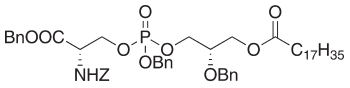
Compound 49.



49

Following the general procedure B, **45** (87.6 mg, 0.304 mmol), **28** (204.0 mg, 0.360 mmol), 1*H*-tetrazole (57.8 mg, 0.825 mmol), TBHP (0.25 mL, 1.50 mmol), CH_2Cl_2(0.75 mL), and THF (1.6 5 mL) were used. The crude mixture was chromatographed (EtOAc/hexane 1:6 to 1:2) to yield **49** (197.7 mg, 0.257 mmol, 85%, colorless oil). A mixture of trans/cis isomers with respect to the benzyloxycarbonylamino group was observed. ^{1}H NMR ($CDCl_3$): δ 7.23−7.30 (20H, m), 6.76 (4H, m), 5.87 ($^1/_2$H, d, J=8.5 Hz), 5.76 ($^1/_2$H, d, J=8.5 Hz), 5.13 (2H, m), 5.07 (2H, m), 4.92 (2H, m), 4.62 (2H, m), 4.55 (1H, m), 4.39 (1H, m), 4.25 (1H, m), 4.21 (1H, m), 4.09 (1H, m), 3.92 (2H, m), 3.84 (1H, m), 3.73 (3H, s). HRMS (ESI, $[M + Na]^+$): Calcd for $C_{42}H_{44}NNaO_{11}P$: 792.2550. Found: 792.2579.

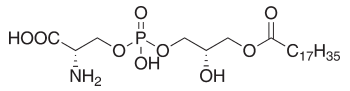
Compound 53.



53

Following the general procedure K, **49** (102.0 mg, 0.133 mmol), CAN (181.6 mg, 0.331 mmol), and CH_3CN-H_2O (5 mL) were used. The crude mixture was chromatographed (EtOAc/hexane 2:3 to 7:3) to yield the intermediate alcohol (75.4 mg, 0.114 mmol, 86%, brown oil). The obtained alcohol (258.1 mg, 0.390 mmol) was acylated with stearoyl chloride (141.9 mg, 0.468 mmol) in CH_2Cl_2 (1.5 mL) in the presence of DMAP (142.6 mg, 1.167 mmol). The crude mixture was chromatographed (EtOAc/hexane 1:3 to 2:3) to yield **53** (290.9 mg, 0.313 mmol, 80%, colorless oil). A mixture of trans/cis isomers with respect to the benzyloxycarbonylamino group was observed. ^{1}H NMR ($CDCl_3$): δ 7.25−7.32 (20H, m), 5.86 ($^1/_2$H, d, J = 8.2 Hz), 5.79 ($^1/_2$H, d, J = 8.2 Hz), 5.16 (2H, m), 5.08 (2H, m), 4.94 (2H, m), 4.57 (3H, m), 4.40 (1H, m), 4.26 (1H, m), 4.18 (2H, m), 4.00 (2H, m), 3.69 (1H, m), 2.24 (2H, m), 1.55 (2H, m), 1.23 (28H, m), 0.85 (3H, t, J = 6.8 Hz). ^{13}C NMR ($CDCl_3$): δ 173.38, 173.35, 168.69, 155.80, 137.51, 137.46, 135.99, 135.37, 135.30, 134.90, 128.65, 128.63, 128.56, 128.44, 128.34, 128.22, 128.13, 128.05, 127.92, 127.81, 127.78, 127.75, 77.20, 74.98, 74.91, 74.85, 74.78, 72.04, 71.95, 69.70, 69.63, 69.54, 67.64, 67.10, 66.48, 66.42, 62.17, 62.12, 54.46, 54.40, 33.99, 31.85, 29.63, 29.59, 29.56, 29.40, 29.29, 29.21, 29.07, 24.77, 22.62, 14.07. ^{31}P NMR ($CDCl_3$): δ −0.37, −0.39. HRMS (ESI, [M + Na]$^+$) Calcd for $C_{53}H_{72}NNaO_{11}P$: 952.4741. Found: 952.4771.

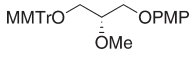
Compound 7.



7 (2*S*-OH)

Following the general procedure L, **53** (63.0 mg, 0.0677 mmol), Pd−C (3.0 mg), and MeOH-AcOH (5 mL) were used. The crude mixture was chromatographed ($CHCl_3$/MeOH/AcOH 9:0:1 to 8:1:1 to 7:2:1), and the product was washed with MeOH to yield **7** (27.0 mg, 0.0514 mmol, 75%, white powder). ^{1}H NMR ($CDCl_3$/CD_3CO_2D 4:1): δ 4.59 (1H, m), 4.34 (1H, m), 4.17 (1H, m), 4.04 (2H, m), 3.90 (2H, m), 3.72(1H, m), 2.23 (2H, m), 1.55(2H, m), 1.08 (28H, m), 0.79 (3H, t, J=7.0 Hz). HRMS (ESI, [M − H]$^-$) Calcd for $C_{24}H_{47}NO_9P$: 524.2988. Found: 524.2971. Anal. Calcd for $C_{24}H_{48}NO_9P + {}^4/_5CF_3CO_2H$: C, 50.57; H, 8.15; N, 2.33. Found: C, 50.31; H, 7.82; N, 2.32. mp 171.5−172.0 °C.

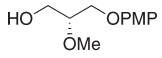
Synthesis of Compound 8. Compound 42.



42

Following the general procedure I, **38** (261.4 mg, 0.556 mmol), NaH (74.2 mg, 1.70 mmol), methyl iodode (396.0 mg, 0.18 mL, 2.80 mmol), and THF (20 mL) were used. The crude mixture was chromatographed (EtOAc/hexane 3:7) to yield **42** (256.0 mg, 0.527 mmol, 95%, yellow oil). ^{1}H NMR ($CDCl_3$): δ 7.42−7.27 (10H, m) 6.80 (8H, m), 4.08 (1H, m), 4.00 (1H, m), 3.77 (3H, s), 3.75 (3H, s), 3.64 (1H, m), 3.43 (3H, s), 3.28 (2H, m). HRMS (ESI, [M + Na]$^+$): Calcd for $C_{31}H_{32}NaO_5$: 507.2147. Found: 507.2151.

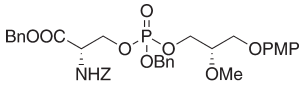
Compound 46.



46

Following the general procedure J, **42** (239.4 mg, 0.493 mmol), *p*-toluenesulfonic acid (9.5 mg, 0.0450 mmol), and CH_2Cl_2-MeOH (5 mL) were used. The crude mixture was chromatographed (EtOAc/hexane 3:7) to yield **46** (83.9 mg, 0.395 mmol, 80%, white solid). ^{1}H NMR ($CDCl_3$): δ 6.82 (4H, m), 4.00 (2H, m), 3.83 (1H, m), 3.72 (1H, m), 3.64 (1H, m), 3.51 (3H, s). HRMS (ESI, [M + Na]$^+$): Calcd for $C_{11}H_{16}NaO_4$: 235.0946. Found: 235.0939. mp 48.5−49.0 °C.

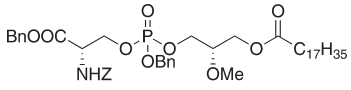
Compound 50.



50

Following the general procedure B, **46** (66.6 mg, 0.314 mmol), **28** (204.0 mg, 0.360 mmol), 1*H*-tetrazole (63.1 mg, 0.900 mmol), TBHP (0.25 mL, 1.50 mmol), CH_2Cl_2 (1.2 mL), and THF (1.8 mL) were used. The crude mixture was chromatographed (EtOAc/hexane 2:3 → 1:1) to yield **50** (209.9 mg, 0.328 mmol, 97%, colorless oil). ^{1}H NMR ($CDCl_3$): δ 7.30 (15H, m), 6.78 (4H, m), 5.92 (1H, m), 5.14 (2H, m), 5.08 (2H, m), 4.97 (2H, m), 4.57 (1H, m), 4.42 (1H, m), 4.26 (1H, m), 4.20 (1H, m), 4.07 (1H, m), 3.90 (2H, m), 3.73 (3H, s), 3.62 (1H, m), 3.40 (3H, m). HRMS (ESI, [M + Na]$^+$): Calcd for $C_{36}H_{40}NnaO_{11}P$: 716.2237. Found: 716.2238.

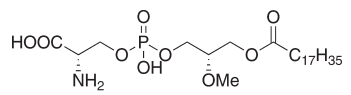
Compound 54.



54

Following the general procedure K, **50** (146.2 mg, 0.211 mmol), CAN (274.1 mg, 0.530 mmol), and CH_3CN-H_2O (3 mL) were used. The crude mixture was chromatographed (EtOAc/hexane 1:1 to 4:1) to yield the intermediate alcohol (94.4 mg, 0.161 mmol, 76%, brown oil). The obtained alcohol (94.4 mg, 0.161 mmol) was acylated with stearoyl chloride (53.6 mg, 0.177 mmol) in CH_2Cl_2 (2.5 mL) in the presence of DMAP (58.9 mg, 0.482 mmol). The crude mixture was chromatographed (EtOAc/hexane 1:3 to 2:3) to yield **54** (112.8 mg, 0.132 mmol, 82%, colorless oil). ^{1}H NMR ($CDCl_3$): δ 7.31 (15H, m), 5.88 (1H, m), 5.16 (2H, m), 5.09 (2H, m), 4.96 (2H, m), 4.59 (1H, m), 4.42 (1H, m), 4.28 (1H, m), 4.14 (1H, m), 4.04 (1H, m), 3.97 (1H, m), 3.47 (1H, m), 3.34 (3H, s), 2.27 (2H, t, J=7.6 Hz), 1.55 (2H, m), 1.23 (28H, m), 0.86 (3H, t, J=7.0 Hz). ^{13}C NMR ($CDCl_3$): δ 173.46, 168.75, 155.86, 136.07, 135.42, 135.36, 134.91, 128.71, 128,62, 128.62, 128.49, 128.28, 128.18, 128.08, 128.01, 127.98, 69.66, 69.60, 67.72, 67.43, 67.15, 66.26, 66.20, 61.83, 61.75, 57.96, 54.46, 34.02, 31.89, 29.66, 29.63, 29.59, 29.45, 29.33, 29.24, 29.09, 24.82, 22,66, 14.09. ^{31}P NMR ($CDCl_3$): δ −0.52, −0.54. HRMS (ESI, [M + Na]$^+$) Calcd for $C_{47}H_{68}NNaO_{11}P$: 876.4428. Found: 876.4439.

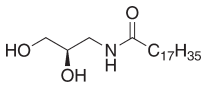
Compound 8.



8 (2*S*-MeO)

Following the general procedure L, **54** (25.3 mg, 0.0296 mmol), Pd−C (3.0 mg), and MeOH-AcOH (5 mL) were used. The crude mixture was chromatographed ($CHCl_3$/MeOH/AcOH 9:0:1 to 8:1:1 to 7:2:1) and the product was washed with MeOH to yield **8** (11.1 mg, 0.0206 mmol, 66%, white powder). ^{1}H NMR ($CDCl_3$/CD_3CO_2D 4:1): δ 4.68 (2H, m), 4.55 (1H, m), 4.32 (3H, m), 4.27 (1H, m), 4.19 (1H, m), 3.84 (1H, m), 3.58 (3H, m), 2.19 (2H, m), 1.59 (2H, m), 1.23 (28H, m), 0.85 (3H, t, J=6.8 Hz). HRMS (ESI, [M − H]$^-$) Calcd for $C_{25}H_{49}NO_9P$: 538.3145. Found: 538.3132. Anal. Calcd for $C_{25}H_{50}NO_9P + {}^1/_2CF_3CO_2H$: C, 52.34; H, 8.53; N, 2.34. Found: C, 52.30; H, 8.36; N, 2.29. mp 179.0−179.5 °C.

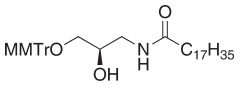
Synthesis of Amide Analogues 9 and 10 (Scheme 3). Synthesis of Compound 9. Compound 55.



55

To a preheated solution (at 50 °C) of (*R*)-3-amino-1,2-propanediol (281.8 mg, 3.09 mmol) and pyridine (1 mL) in DMF (20 mL), a solution of stearoyl chloride (1.030 g, 3.042 mmol) in DMF (10 mL) was added and the whole was stirred for 3 h. The organic solvent was evaporated, and the residue was chromatographed ($CHCl_3$/MeOH 1:0 to 9:1) to yield **55** (638.2 mg, 1.79 mmol, 58%, white solid). ^{1}H NMR ($CDCl_3$): δ 4.74 (1H, bs), 3.74 (1H, m), 3.54 (2H, m), 3.40 (2H, m), 2.20 (2H, t, J = 7.6 Hz), 1.54 (2H, m), 1.23 (28H, m), 0.86 (3H, t, J = 6.8 Hz). HRMS (ESI, $[M + Na]^+$): Calcd for $C_{21}H_{43}NNaO_3$: 380.3141. Found: 380.3141. mp 109.0−109.5 °C.

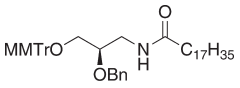
Compound 56.



56

To a preheated solution (at 50 °C) of **55** (603.5 mg, 1.69 mmol), TBAI (198.9 mg, 0.537 mmol), and DIPEA (0.88 mL, 654.4 mg, 5.06 mmol) in THF (20 mL), 4-methoxy tritylchloride (576.5 mg, 1.87 mmol) was added and the whole was heated at 50 °C with stirring for 2 h. The whole was cooled to room temperature, and the reaction was quenched with 3N aqueous HCl. The obtained organic layer was washed with 3N aqueous HCl and brine and was dried over Na_2SO_4. The solvent was evaporated, and the residue was chromatographed (EtOAc/hexane 1:2) to yield **56** (877.8 mg, 1.394 mmol, 83%, yellow oil). ^{1}H NMR ($CDCl_3$): δ 7.39 (4H, m), 7.29−7.20 (8H, m), 6.82 (2H, m), 5.63 (1H, m), 3.87 (1H, m), 3.78 (3H, s), 3.54 (1H, m), 3.24−3.10 (3H, m), 2.05 (2H, t, J = 7.6 Hz), 1.52 (2H, m), 1.23 (28H, m), 0.86 (3H, t, J = 6.8 Hz). HRMS (ESI, $[M + H]^+$): Calcd for $C_{41}H_{59}NNaO_4$: 652.4342. Found: 652.4368. mp 47.5−48.0 °C.

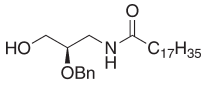
Compound 57.



57

Following the general procedure H, **56** (1.53 g, 2.43 mmol), NaH (127.1 mg, 2.91 mmol), benzyl bromide (435.9 mg, 2.55 mmol), and THF (40 mL) were used. The crude mixture was chromatographed (EtOAc/hexane 1:10 to 1:5) to yield **57** (1.669 g, 2.32 mmol, 95%, yellow oil). ^{1}H NMR ($CDCl_3$): δ 7.20−7.35 (17H, m), 6.82 (2H, m), 5.72 (1H, m), 4.57 (2H,dd, J = 15.2 Hz, 5.6 Hz,), 3.78 (3H, s) 3.55−3.70 (3H, m), 3.48 (1H, m), 3.29 (1H, m), 2.16 (2H, t, J = 7.6 Hz), 1.57 (2H, m), 1.23 (28H, m), 0.86 (3H, t, J = 6.8 Hz). HRMS (ESI, $[M + Na]^+$): Calcd for $C_{48}H_{65}NNaO_4$: 742.4811. Found: 742.4813. mp 66.5−67.0 °C.

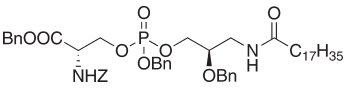
Compound 59.



59

Following the general procedure J, **57** (357.5 mg, 0.497 mmol), *p*-toluenesulfonic acid (8.6 mg, 0.0410 mmol), and CH_2Cl_2-MeOH (5 mL) were used. The crude mixture was chromatographed (EtOAc/hexane 1:1 to 3:2) to yield **59** (179.1 mg, 0.400 mmol, 80%, white solid). ^{1}H NMR ($CDCl_3$): δ 7.35−7.29 (5H, m), 5.73 (1H, m), 4.57 (2H, dd, J = 15.2 Hz, 6.0 Hz), 3.70−3.55 (3H, m), 3.52−3.45 (1H, m), 3.36−3.28 (1H, m), 2.15 (2H, t, J = 7.6 Hz), 1.59 (2H, m), 1.23 (28H, m), 0.86 (3H, t, J = 7.0 Hz). HRMS (ESI, $[M + Na]^+$): Calcd for $C_{28}H_{49}NNaO_3$: 470.3610. Found: 470.3615. mp 109.0−109.5 °C.

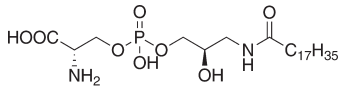
Compound 61.



61

Following the general procedure B, **59** (45.0 mg, 0.0991 mmol), **28** (67.7 mg, 0.119 mmol), 1*H*-tetrazole (23.3 mg, 0.332 mmol), TBHP (0.08 mL, 0.480 mmol), CH_2Cl_2 (0.4 mL), and THF (0.6 mL) were used. The crude mixture was chromatographed (EtOAc/hexane 3:7) to yield **61** (71.4 mg, 0.328 mmol, 77%, colorless oil). ^{1}H NMR ($CDCl_3$): δ 7.31−7.25 (20H, m), 6.00 (1H, dd, J = 8.0 Hz, 4.0 Hz), 5.15 (2H, m), 5.08 (2H, m), 4.96 (2H, m), 4.62−4.53 (2H, m), 4.47−4.39 (2H, m), 4.30 (1H, m), 4.24 (1H, m), 4.06−3.88 (2H, m), 3.64−3.55 (1H, m), 3.41−3.32 (1H, m), 3.30−3.23 (1H, m), 2.00 (2H, m), 1.55 (2H, m), 1.23 (28H, m), 0.86 (3H, t, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$): δ 173.41, 173.30, 170.13, 168.73, 155.79, 137.53, 137.33, 136.31, 135.99, 135.18, 134.89, 128.73, 128.61, 128.57, 128.47, 128.38, 128.34, 128.29, 128.19, 128.04, 127.98, 127.94, 127.91, 127.85, 127.77, 127.44, 75.37, 71.90, 71.82, 69.73, 67.77, 67.13, 66.77, 65.01, 62.30, 54.50, 54.42, 40.48, 39.10, 39.03, 36.49, 33.85, 31.86, 29.65, 29.60, 29.47, 29.44, 29.30, 29.26, 25.56, 24.72, 22.63, 14.07. ^{31}P NMR ($CDCl_3$): δ −0.30. HRMS (ESI, $[M + Na]^+$): Calcd for $C_{53}H_{73}N_2NaO_{10}P$: 951.4901. Found: 951.4869.

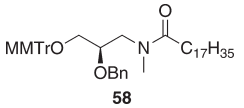
Compound 9.



9

Following the general procedure L, **61** (31.8 mg, 0.0343 mmol), Pd−C (3.0 mg), and MeOH-AcOH (5 mL) were used. The crude mixture was chromatographed ($CHCl_3$/MeOH/AcOH 9:0:1 to 8:1:1 to 7:2:1) to yield **9** (14.0 mg, 0.0267 mmol, 78%, white powder). ^{1}H NMR ($CDCl_3$/CD_3CO_2D 4:1): δ 4.39 (3H, m), 3.89 (3H, m), 3.16 (3H, m), 2.13 (2H, m), 1.49 (2H, m), 1.17 (28H, m), 0.79 (3H, t, J = 6.8 Hz). HRMS (ESI, $[M - H]^-$): Calcd for $C_{24}H_{48}N_2O_8P$: 523.3148. Found: 523.3154. Anal. Calcd for $C_{24}H_{49}N_2O_8P + {}^5/_6CF_3CO_2H$: C, 49.75; H, 8.11; N, 4.52. Found: C, 49.84; H, 8.33; N, 4.73. mp 180.5−181.0 °C.

Synthesis of Compound 10. Compound 58.

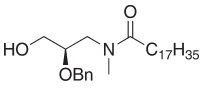


58

Following the general procedure I, **57** (338.6 mg, 0.470 mmol), NaH (33.8 mg, 0.740 mmol), methyl iodode (80.1 mg, 0.564 mmol), and THF (20 mL) were used. The crude mixture was chromatographed (EtOAc/hexane 3:7) to yield **58** (220.9 mg, 0.301 mmol, 64%, yellow oil). ^{1}H NMR ($CDCl_3$): δ 7.47−7.41 (4H, m), 7.35−7.20 (13H, m), 6.82 (2H, m), 4.70−4.30 (2H, m), 3.87 (1H, m), 3.76 (3H, m), 3.55 (1H, m), 3.45 (1H, m), 3.40−3.18 (2H, m), 2.90−2.81 (3H, m), 2.30−2.16 (2H, m),

1.54 (2H, m), 1.23 (28H, m), 0.86 (3H, t, $J = 6.8$ Hz). HRMS (ESI, $[M + Na]^+$): Calcd for $C_{49}H_{67}NNaO_4$: 756.4968. Found: 756.4969.

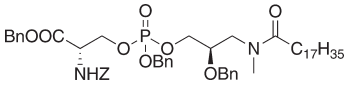
Compound 60.



60

Following the general procedure J, **58** (221.8 mg, 0.302 mmol), *p*-toluenesulfonic acid (15.4 mg, 0.0730 mmol), and CH_2Cl_2-MeOH (5 mL) were used. The crude mixture was chromatographed (EtOAc/hexane 1:4→1:3) to yield **60** (122.3 mg, 0.265 mmol, 88%, white solid). ^{1}H NMR ($CDCl_3$): δ 7.35−7.23 (5H, m), 4.58 (2H, m), 3.90 (1H, m), 3.64 (1H, m), 3.55 (1H, m), 3.42 (1H, m), 3.30 (1H, m), 3.06 (3H, s), 2.31 (2H, m), 1.54 (2H, m), 1.23 (28H, m), 0.86 (3H, t, $J = 6.8$ Hz). HRMS (ESI, $[M + Na]^+$): Calcd for $C_{29}H_{51}NNaO_3$: 484.3767. Found: 484.3767. mp 34.0−34.5 °C.

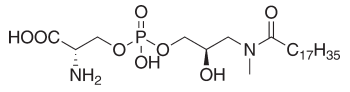
Compound 62.



62

Following the general procedure B, using **60** (45.5 mg, 0.0985 mmol), **28** (67.6 mg, 0.119 mmol), 1*H*-tetrazole (20.6 mg, 0.294 mmol), TBHP (0.08 mL, 0.48 mmol), CH_2Cl_2 (0.4 mL), and THF (0.6 mL). The crude mixture was chromatographed (EtOAc/hexane 3:7) to yield **62** (56.9 mg, 0.0594 mmol, 60%, colorless oil). A mixture of trans/cis isomers with respect to the benzyloxycarbonyl-amino group was observed. ^{1}H NMR ($CDCl_3$): δ 7.31−7.25 (20H, m), 6.11 ($^1/_2$H, d, J=4.0 Hz), 6.01 ($^1/_2$H, d, J=4.0 Hz), 5.15 (2H, m), 5.08(2H, m), 4.96 (2H, m), 4.58 (2H, m), 4.40 (2H, m), 4.27 (1H, m), 4.10 (1H, m), 3.90 (2H, m), 3.57 (1H, m), 3.15 (1H, m), 2.87−2.75 (3H, m), 2.15 (2H, m), 1.55 (2H, m), 1.23 (28H, m), 0.86 (3H, t, $J = 7.0$ Hz). ^{13}C NMR ($CDCl_3$): δ 173.49, 173.18, 169.71, 168.72, 156.17, 155.90, 137.82, 137.33, 136.26, 136.05, 135.25, 134.94, 128.61, 128.56, 128.51, 128.44, 128.40, 128.36, 128.29, 128.25, 128.16, 128.0, 128.01, 127.95, 127.90, 127.87, 127.79, 127.72, 127.44, 75.94, 73.02, 72.05, 71.89, 69.53, 67.54, 66.77, 65.44, 64.89, 62.07, 55.14, 54.43, 49.96, 49.40, 37.57, 33.85, 33.60, 33.41, 31.82, 29.61, 29.56, 29.47, 29.39, 29.27, 29.21, 24.80, 24.72, 22.60, 14.04. ^{31}P NMR ($CDCl_3$): δ −0.16. HRMS (ESI, $[M + Na]^+$): Calcd for $C_{54}H_{75}N_2NaO_{10}P$: 965.5057. Found: 965.5090.

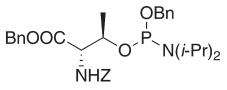
Compound 10.



10

Following the general procedure L, **62** (35.0 mg, 0.0371 mmol), Pd−C (3.0 mg), and MeOH-AcOH (5 mL) were used. The crude mixture was chromatographed ($CHCl_3$/MeOH/AcOH 9:0:1 to 8:1:1 to 7:2:1) and the product was washed with MeOH to yield **10** (13.2 mg, 0.0245 mmol, 66%, white powder). ^{1}H NMR ($CDCl_3$/CD_3CO_2D 4:1): δ 4.40 (3H, m), 3.96 (3H, m), 3.50 (3H, m), 3.11−2.75 (3H, m), 2.27−2.13 (2H, m), 1.49 (2H, m), 1.17 (28H, m), 0.79 (3H, t, $J = 6.8$ Hz). HRMS (ESI, $[M - H]^-$): Calcd for $C_{24}H_{48}N_2O_8P$: 537.3305. Found: 537.3326. Anal. Calcd for $C_{24}H_{49}N_2O_8P + CF_3CO_2H$: C, 49.69; H, 8.03; N, 4.29. Found: C, 49.84; H, 8.30; N, 4.00. mp 174.0−174.5 °C.

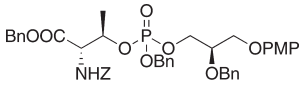
Synthesis of LysoPT Analogues 11 and 12 (Scheme 4). Synthesis of Compound 11 (LysoPT). Compound 65.



65

Following the general procedure A, *N*-Z-L-threonine benzyl ester (**63**, 515.3 mg, 1.50 mmol), **27** (511.4 mg, 1.55 mmol), 1*H*-tetrazole (105.1 mg, 1.50 mmol), CH_2Cl_2 (12 mL), and THF (3 mL) were used. The crude mixture was chromatographed (EtOAc/hexane/Et_3N 100:0:3 to 20:80:3) to yield **65** (609.0 mg, 1.05 mmol, 70%, colorless oil). ^{1}H NMR (CD_2Cl_2): δ 7.33 (15H, m), 5.58 ($^1/_2$H, d, J=9.2 Hz), 5.48 ($^1/_2$H, d, J=9.2 Hz), 5.09 (4H, m), 4.67 (1H, m), 4.57 (2H, m), 4.33 (1H, m), 3.57 (2H, m), 1.28 (3H, m), 1.14 (12H, m). ^{13}C NMR ($CDCl_3$): δ 171.38, 170.29, 157.01, 156.65, 142.02, 138.26, 138.19, 136.87, 136.70, 135.95, 135.88, 135.55, 128.51, 128.47, 128.43, 128.39, 128.28, 128.24, 128.19, 128.12, 128.06, 128.01, 127.90, 127.59, 127.43, 126.94, 126.77, 67.42, 67.15, 66.97, 66.82, 66.77, 66.59, 65.70, 65.66, 64.11, 46.44, 20.08, 18.74, 18.72. ^{31}P NMR ($CDCl_3$): δ 2.79, 1.60. HRMS (ESI, $[M + Na]^+$): Calcd for $C_{32}H_{41}N_2NaO_3P$: 603.2600. Found: 603.2586.

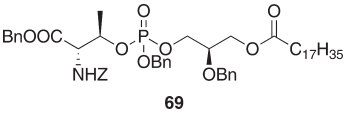
Compound 67.



67

Following the general procedure B, **43** (153.6 mg, 0.507 mmol), **65** (344.2 mg, 0.594 mmol), 1*H*-tetrazole (105.8 mg, 1.51 mmol), TBHP (0.42 mL, 2.52 mmol), CH_2Cl_2 (2 mL), and THF (3 mL) were used. The crude mixture was chromatographed (EtOAc/hexane 1:3) to yield **67** (392.2 mg, 0.501 mmol, 99%, colorless oil). ^{1}H NMR ($CDCl_3$): δ 7.30 (20H, m), 6.75 (4H, m), 5.09 (2H, m), 5.03 (2H, m), 4.93 (3H, m), 4.44 (1H, m), 4.16 (1H, m), 4.09 (1H, m), 3.93 (2H, m), 3.84 (1H, m), 3.73 (3H, m), 1.33 (3H, m). ^{13}C NMR ($CDCl_3$): δ 169.05, 169.02, 156.21, 153.74, 152.21, 152.19, 137.52, 1237.51, 135.78, 135.77, 135.34, 135.31, 135.27, 135.25, 134.71, 134.67, 128.22, 128.16, 128.05, 128.00, 127.84, 127.71, 127.57, 127.52, 127.49, 127.45, 115.17, 115.14, 114.28, 77.60, 77.32, 77.20, 77.00, 76.68, 75.36, 75.28, 75.24, 75.17, 75.12, 75.06, 71.91, 71.89, 71.81, 69.12, 69.10, 69.06, 69.05, 68.10, 67.31, 67.29, 66.86, 66.37, 66.31, 66.25, 65.42, 61.80, 58.33, 58.26, 58.20, 55.22, 18.12, 14.95. ^{31}P NMR ($CDCl_3$): δ −1.34. HRMS (ESI, $[M + Na]^+$): Calcd for $C_{43}H_{46}NNaO_{11}P$: 806.2706. Found: 806.2692.

Compound 69.

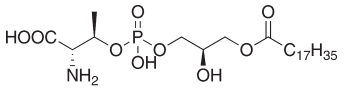


69

Following the general procedure K, **67** (250.1 mg, 0.319 mmol), CAN (441.4 mg, 0.805 mmol), and CH_3CN-H_2O (3 mL) were used. The crude mixture was chromatographed (EtOAc/hexane 1:1 to 4:1) to yield the intermediate alcohol (154.6 mg, 0.228 mmol, 71%, brown oil). The obtained alcohol (154.6 mg, 0.228 mmol) was acylated with stearoyl chloride (76.1 mg, 0.251 mmol) in CH_2Cl_2 (2.5 mL), in the presence of DMAP (82.1 mg, 0.672 mmol). The crude mixture was chromatographed (EtOAc/hexane 1:3 to 2:3) to yield **69** (192.7 mg, 0.204 mmol, 90%, colorless oil). ^{1}H NMR ($CDCl_3$): δ 7.31 (20H, m), 5.88 (1H, m), 5.16 (2H, m), 5.09 (2H, m), 4.96 (2H, m), 4.59 (1H, m),

4.42 (1H, m), 4.28 (1H, m), 4.14 (1H, m), 4.04 (1H, m), 3.97 (1H, m), 3.47 (1H, m), 3.34 (3H, s), 2.27 (2H, t, J = 7.6 Hz), 1.55 (2H, m), 1.36 ($^3/_2$H, d, J = 6.4 Hz), 1.31 ($^3/_2$H, d, J = 6.4 Hz), 1.23 (28H, m), 0.86 (3H, t, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$): δ 173.38, 173.22, 168.46, 168.43, 165.50, 155.85, 137.71, 136.15, 135.60, 135.54, 134.96, 129.83, 129.80, 128.59, 128.57, 128.48, 128.35, 128.21, 128.13, 128.09, 127.89, 127.86, 127.84, 127.81, 127.78, 77.21, 75.91, 75.85, 75.76, 75.10, 75.02, 74.95, 72.11, 72.05, 74.95, 72.11, 72.05, 69.63, 69.61, 69.58, 69.55, 67.51, 67.50, 67.10, 66.54, 66.49, 62.33, 62.29, 58.61, 34.06, 33.99, 31.90, 29.68, 29.64, 29.61, 29.46, 29.34, 29.27, 29.13, 24.83, 24.67, 18.49, 18.44, 14.10. ^{31}P NMR ($CDCl_3$): δ −1.29, −1.35. HRMS (ESI, [M + Na]$^+$): Calcd for $C_{57}H_{74}NNaO_{11}P$: 966.4897. Found: 966.4909.

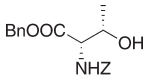
Compound 11 (LysoPT).



11 (lysoPT)

Following the general procedure L, **69** (46.8 mg, 0.0496 mmol), Pd−C (3.0 mg), and MeOH-AcOH (5 mL) were used. The crude mixture was chromatographed ($CHCl_3$/MeOH/AcOH 9:0:1 to 8:1:1 to 7:2:1), and washed with MeOH to yield **11** (17.7 mg, 0.0327 mmol, 66%, white powder). ^{1}H NMR ($CDCl_3$/CD_3CO_2-D 4:1): δ 4.79 (1H, m), 4.04−3.80 (6H, m), 2.24 (2H, m), 1.49 (2H, m), 1.17 (35H, m), 0.78 (3H, t, J = 6.8 Hz). HRMS (ESI, [M − H]$^-$): Calcd for $C_{25}H_{49}NO_9P$: 538.3145. Found: 538.3141. Anal. Calcd for $C_{25}H_{50}NO_9P + CF_3CO_2H$: C, 49.46; H, 7.85; N, 2.42. Found: C, 49.61; H, 7.85; N, 2.42. mp 176.5−177.0 °C.

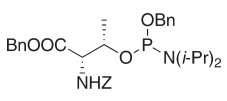
Synthesis of Compound 12 (LysoPalloT). Compound 64.



64

Following the general procedure E, L-*allo*-threonine (101.4 mg, 0.851 mmol), benzyl chloroformate (174.3 mg, 1.021 mmol), saturated aqueous $NaHCO_3$ (10 mL), and THF (10 mL) were used. The crude mixture was chromatographed ($CHCl_3$/MeOH 1:0 to 9:1) to yield *N*-Z-L-*allo*-threonine (221.3 mg, 0.874 mmol, quantitative yield, pale-yellow oil). The following benzylation was carried out in accordance with the general procedure F, which involved the intermediate alcohol (221.3 mg, 0.874 mmol), benzyl bromide (179.4 mg, 1.049 mmol), triethylamine (0.43 mL, 2.621 mmol), and THF (10 mL). The crude mixture was chromatographed (EtOAc/hexane 1:3) to yield **64** (213.0 mg, 0.620 mmol, 73% (in 2 steps), white solid). ^{1}H NMR ($CDCl_3$): δ 7.33 (10H, m), 5.65 (1H, m), 5.18 (1H, m), 5.10 (2H, m), 4.47 (1H, m), 4.15 (1H, m), 1.13 (3H, d, J = 6.4 Hz). HRMS (ESI, [M + Na]$^+$): Calcd for $C_{19}H_{21}NNaO_5$: 366.1317. Found: 366,1317. mp 74.0−74.5 °C.

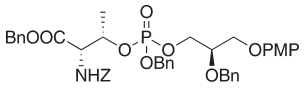
Compound 66.



66

Following the general procedure A, **64** (197.7 mg, 0.576 mmol), **27** (190.0 mg, 0.576 mmol), 1*H*-tetrazole (40.3 mg, 0.576 mmol), CH_2Cl_2 (5 mL), and THF (1.2 mL) were used. The crude mixture was chromatographed (EtOAc/hexane/Et_3N 100:0:3 to 20:80:3) to yield **66** (175.7 mg, 0.303 mmol, 53%, colorless oil). ^{1}H NMR (CD_2Cl_2): δ 7.33 (15H, m), 6.19 ($^1/_2$H, m), 5.61 ($^1/_2$H, m), 5.22−5.09 (4H, m), 4.76 (1H, m), 4.59 (2H, m), 4.38 (1H, m), 4.28 (1H, m) 3.58 (2H, m), 1.33 (3H, m), 1.14 (12H, m). HRMS (ESI, [M + Na]$^+$): Calcd for $C_{32}H_{41}N_2NaO_3P$: 603.2600. Found: 603.2598.

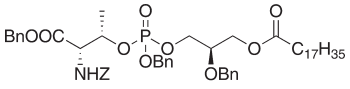
Compound 68.



68

Following the general procedure B, **43** (100.8 mg, 0.333 mmol), **66** (160.9 mg, 0.277 mmol), 1*H*-tetrazole (48.5 mg, 0.693 mmol), TBHP (0.11 mL, 0.66 mmol), CH_2Cl_2 (1 mL), and THF (1.5 mL) were used. The crude mixture was chromatographed (EtOAc/hexane 1:2) to yield **68** (85.0 mg, 0.108 mmol, 39%, colorless oil). ^{1}H NMR ($CDCl_3$): δ 7.27 (20H, m), 6.75 (4H, m), 6.12 (1H, m), 5.13−4.93 (6H, m), 4.73 (1H, m), 4.63 (2H, m), 4.49 (1H, m), 4.22 (1H, m), 4.09 (1H, m), 3.99−3.85 (3H, m), 3.73 (3H, m), 1.24 (3H, m). HRMS (ESI, [M + Na]$^+$): Calcd for $C_{43}H_{46}NNaO_{11}P$: 806.2706. Found: 806.2718.

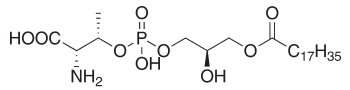
Compound 70.



70

Following the general procedure K, **68** (208.5 mg, 0.271 mmol), CAN (371.3 mg, 0.677 mmol), and CH_3CN-H_2O (6 mL) were used. The crude mixture was chromatographed (EtOAc/hexane 1:1 to 4:1) to yield the intermediate alcohol (145.5 mg, 0.215 mmol, 79%, brown oil). The obtained alcohol (145.5 mg, 0.215 mmol) was acylated with stearoyl chloride (78.2 mg, 0.258 mmol) in CH_2Cl_2 (5 mL) in the presence of DMAP (78.8 mg, 0.645 mmol). The crude product was chromatographed (EtOAc/hexane 1:3 to 2:3) to yield **70** (180.5 mg, 0.191 mmol, 89%, colorless oil). ^{1}H NMR ($CDCl_3$): δ 7.28 (20H, m), 6.09(1H, m), 5.15 (2H, m), 5.07 (2H, m), 4.97 (2H, m), 4.73 (1H, m), 4.56 (2H, m), 4.16 (1H, m), 4.07 (2H, m), 3.95 (1H, m), 3.70 (1H, m), 2.24 (2H, t, J = 7.6 Hz), 1.55 (2H, m), 1.34 (3H, t, J = 6.4 Hz), 1.23 (28H, m), 0.86 (3H, t, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$): δ 173.39, 168.42, 155.84, 137.68, 137.54, 136.13, 135.52, 134.94, 128.59, 128.56, 128.47, 128.38, 128.35, 128.13, 128.09, 127.88, 127.81, 127.78, 75.82, 75.06, 74.99, 74.91, 72.09, 72.03, 69.59, 67.49, 67.09, 66.46, 62.27, 58.59, 34.05, 31.89, 29.67, 29.63, 29.60, 29.45, 29.33, 29.26, 29.12, 24.81, 22.66, 14.10. ^{31}P NMR ($CDCl_3$): δ −1.29, −1.35. HRMS (ESI, [M + Na]$^+$): Calcd for $C_{57}H_{74}NNaO_{11}P$: 966.4897. Found: 966.4916.

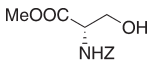
Compound 12 (LysoPalloT).



12 (lysoPalloT)

Following the general procedure L, **70** (41.0 mg, 0.0435 mmol), Pd−C (3.0 mg), and MeOH-AcOH (5 mL) were used. The crude mixture was chromatographed ($CHCl_3$/MeOH/AcOH 9:0:1 to 8:1:1 to 7:2:1) to yield **12** (12.6 mg, 0.0159 mmol, 37%, white powder). ^{1}H NMR ($CDCl_3$/CD_3CO_2D 4:1): δ 4.23 (2H, m), 4.05 (5H, m), 2.24 (2H, m), 1.52(2H, m), 1.17 (31H, m), 0.79 (3H, t, J = 6.8 Hz). HRMS (ESI, [M − H]$^-$): Calcd for $C_{25}H_{49}NO_9P$: 538.3145. Found: 538.3137. mp 179.0−179.5 °C.

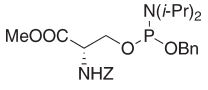
Synthesis of Compounds 13 and 14 (Scheme 5). Synthesis of Compound 13. Compound 71.



71

Following the general procedure E, L-serine methyl ester hydrochloride (1.56 g, 10.0 mmol), benzyl chloroformate (2.05 g, 12.0 mmol), saturated aqueous $NaHCO_3$ (50 mL), and THF (50 mL) were used. The crude mixture was chromatographed (EtOAc/hexane 1:1) to yield **71** (2.143 g, 8.462 mmol, 84.6%, colorless oil). ^{1}H NMR ($CDCl_3$): δ 7.33 (5H, m), 5.69 (1H, m), 5.10 (2H, m), 4.43 (1H, m), 3.96 (1H, m), 3.90 (1H, m), 3.76 (3H, s). HRMS (ESI, $[M + Na]^+$): Calcd for $C_{12}H_{15}NNaO_5$: 276.0848. Found: 276.0849.

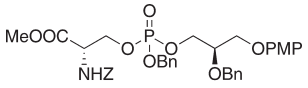
Compound 72.



72

Following the general procedure A, **71** (199.1 mg, 0.603 mmol), **27** (201.9 mg, 0.612 mmol), 1*H*-tetrazole (42.1 mg, 0.603 mmol), CH_2Cl_2 (5 mL), and THF (1.2 mL) were used. The crude mixture was chromatographed (EtOAc/hexane/Et_3N 100:0:3 to 20:80:3) to yield **72** (173.7 mg, 0.355 mmol, 59%, colorless oil). A mixture of trans/cis isomers with respect to the benzyloxycarbonylamino group was observed. ^{1}H NMR (CD_2Cl_2): δ 7.31 (15H, m), 5.80 ($^1/_2$H, m), 5.62 ($^1/_2$H, m), 5.10 (2H, m), 4.65 (2H, m). 4.46 (2H, m), 4.10 (1H, m), 3.87 (1H, m), 3.71 (3H, m), 3.60 (2H, m), 1.16 (12H, m). HRMS (ESI, $[M + Na]^+$): Calcd for $C_{25}H_{35}N_2NaO_6P$: 513.2130. Found: 513.2109.

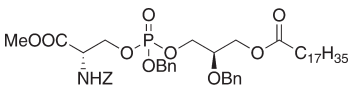
Compound 73.



73

Following the general procedure B, **43** (115.1 mg, 0.380 mmol), **72** (156.3 mg, 0.319 mmol), 1*H*-tetrazole (65.4 mg, 0.934 mmol), TBHP (0.16 mL, 0.96 mmol), CH_2Cl_2 (2 mL), and THF (2 mL) were used. The crude mixture was chromatographed (EtOAc/hexane 1:2 to 1:1) to yield **73** (145.3 mg, 0.209 mmol, 66%, colorless oil). ^{1}H NMR ($CDCl_3$): δ 7.29 (15H, m), 6.77 (4H, m), 5.82 (1H, m), 5.08 (2H, m), 4.99 (2H, m), 4.66 (2H, m), 4.51 (1H, m), 4.38 (1H, m), 4.26 (2H, m), 4.13 (1H, m), 3.92 (2H, m), 3.86 (1H, m), 3.73 (3H, m), 3.69 (3H, m). HRMS (ESI, $[M + Na]^+$): Calcd for $C_{36}H_{40}NNaO_{11}P$: 716.2237. Found: 716.2238.

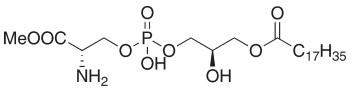
Compound 74.



74

Following the general procedure K, **73** (127.0 mg, 0.183 mmol), CAN (258.7 mg, 0.472 mmol), and CH_3CN-H_2O (6 mL) were used. The crude mixture was chromatographed (EtOAc/hexane 1:1 to 4:1) to yield the intermediate alcohol (79.9 mg, 0.136 mmol, 74%, brown oil). The obtained alcohol (79.9 mg, 0.136 mmol) was acylated with stearoyl chloride (49.4 mg, 0.163 mmol) in CH_2Cl_2 (5 mL) in the presence of DMAP (33.2 mg, 0.272 mmol). The crude mixture was chromatographed (EtOAc/hexane 1:3 to 1:2) to yield **74** (98.3 mg, 0.115 mmol, 85%, colorless oil) ^{1}H NMR ($CDCl_3$): δ 7.32 (15H, m), 5.80 (1H, m), 5.09 (2H, m), 5.00 (2H, m), 4.58 (2H, m), 4.37 (1H, m), 4.24 (1H, m), 4.18 (1H, m), 4.10 (1H, m), 4.01 (1H, m), 3.70 (4H, m), 2.25 (2H, t, J = 7.6 Hz), 1.53 (2H, m), 1.23 (28H, m), 0.86 (3H, t, J = 7.2 Hz). ^{13}C NMR ($CDCl_3$): δ 173.40, 169.27, 155.81, 137.55, 137.50, 136.06, 135.42, 135.36, 128.72, 128.70, 128.62, 128.50, 128.41, 128.39, 128.19, 128.09, 127.96, 127.89, 127.86, 127.83, 77.21, 75.01, 74.97, 74.94, 74.90, 72.11, 69.73, 69.69, 67.38, 67.16, 66.60, 66.54, 66.48, 65.82, 62.21, 54.37, 54.30, 52.81, 34.05, 31.89, 29.67, 29.63, 29.60, 29.45, 29.33, 29.25, 29.12, 24.82, 22.66, 15.24, 14.09. ^{31}P NMR ($CDCl_3$): δ −0.34. HRMS (ESI, $[M + Na]^+$) Calcd for $C_{47}H_{68}NNaO_{11}P$: 876.4428. Found: 876.4404.

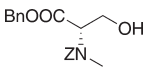
Compound 13.



13

Following the general procedure L, **74** (28.5 mg, 0.0333 mmol), Pd−C (3.0 mg), and MeOH-AcOH (5 mL) were used. The crude mixture was chromatographed ($CHCl_3$/MeOH/AcOH 9:0:1 to 8:1:1 to 7:2:1) to yield **13** (10.3 mg, 0.0190 mmol, 57%, white powder). ^{1}H NMR ($CDCl_3$/CD_3CO_2D 4:1): δ 4.55 (1H, m), 4.38 (2H, m), 4.04 (5H, m), 3.78 (3H, s), 2.25 (2H, m), 1.52 (2H, m), 1.17 (28H, m), 0.78 (3H, t, J = 6.8 Hz). HRMS (ESI, $[M - H]^-$): Calcd for $C_{25}H_{49}NO_9P$: 538.3145. Found: 538.3127. mp 150.5−151.0 °C.

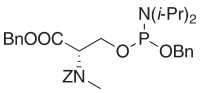
Synthesis of Compound 14. Compound 76.



76

Following the general procedure F, **75** (524.6 mg, 2.07 mmol), benzyl bromide (576.0 mg, 3.37 mmol), TBAI (229.4 mg, 0.621 mmol), triethylamine (1.05 mL, 6.21 mmol), and THF (30 mL) were used. The crude mixture was chromatographed (EtOAc/hexane 1:3 to 1:1) to yield **76** (545.8 mg, 1.59 mmol, 56% (2 steps), white solid). ^{1}H NMR ($CDCl_3$): δ 7.32 (10H, m), 5.22−4.98 (4H, m), 4.55−4.48 (1H, m), 4.08 (1H, m), 3.91−3.85 (1H, m), 2.95 (3H, m). HRMS (ESI, $[M + Na]^+$): Calcd for $C_{19}H_{21}NNaO_5$: 366.1317. Found: 363.1329.

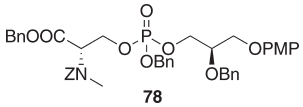
Compound 77.



77

Following the general procedure A, **76** (172.8 mg, 0.524 mmol), **27** (162.3 mg, 0.492 mmol), 1*H*-tetrazole (35.1 mg, 0.500 mmol), CH_2Cl_2 (4 mL), and THF (1 mL) were used. The crude mixture was chromatographed (EtOAc/hexane/Et_3N 100:0:3 to 20:80:3) to yield **77** (184.2 mg, 0.318 mmol, 65%, colorless oil). ^{1}H NMR (CD_2Cl_2): δ 7.31 (15H, m), 5.20−4.94 (5H, m), 4.83 (1H, m), 4.67 (1H, m), 4.09 (1H, m), 3.59 (2H, m), 3.40 (1H, m), 3.00−2.89 (3H, m), 1.20 (12H, m). HRMS (ESI, $[M + Na]^+$): Calcd for $C_{32}H_{41}N_2NaO_6P$: 603.2600. Found: 603.2600.

Compound 78.

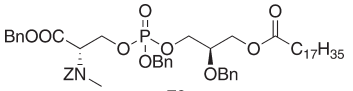


78

Following the general procedure B, **43** (96.0 mg, 0.317 mmol), **77** (153.2 mg, 0.264 mmol), 1*H*-tetrazole (55.5 mg, 0.793 mmol),

TBHP (0.25 mL, 1.50 mmol), CH_2Cl_2 (2 mL), and THF (1.5 mL) were used. The crude mixture was chromatographed (EtOAc/hexane 1:3 to 1:1) to yield **78** (173.9 mg, 0.222 mmol, 84%, colorless oil). ^{1}H NMR ($CDCl_3$): δ 7.27 (20H, m), 6.76 (4H, m), 5.16−4.94 (6H, m), 4.81 (1H, m), 4.64 (2H, m), 4.42 ($^3/_2$H, m), 4.22 ($^3/_2$H, m), 4.11 (1H, m), 3.94 (2H, m), 3.86 (1H, m), 3.73 (3H, m), 2.89 (3H, s). HRMS (ESI, $[M + Na]^+$): Calcd for $C_{43}H_{46}NNaO_{11}P$: 806.2706. Found: 806.2707.

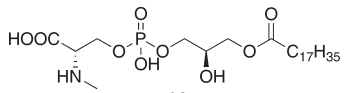
Compound 79.



79

Following the general procedure K, **78** (107.3 mg, 0.134 mmol), CAN (184.3 mg, 0.336 mmol), and CH_3CN-H_2O (2 mL) were used. The crude mixture was chromatographed (EtOAc/hexane 1:1 to 4:1) to yield the intermediate alcohol (53.5 mg, 0.0773 mmol, 58%, brown oil). The obtained alcohol (53.5 mg, 0.0773 mmol) was acylated with stearoyl chloride (28.1 mg, 0.0928 mmol) in CH_2Cl_2 (5 mL) in the presence of DMAP (18.9 mg, 0.155 mmol). The crude mixture was chromatographed (EtOAc/hexane 1:3) to yield **79** (52.8 mg, 0.0558 mmol, 72%, colorless oil). ^{1}H NMR ($CDCl_3$): δ 7.29 (20H, m), 5.14−4.96 (6H, m), 4.82 (1H, m), 4.57 (2H, m), 4.43 (2H, m), 4.17 (1H, m), 4.07 (3H, m), 3.71 (2H, m), 2.90 (3H, m), 2.25 (2H, m), 1.55 (2H, m), 1.23 (28H, m), 0.86 (3H, t, J = 6.8 Hz). ^{13}C NMR ($CDCl_3$): δ 173.40, 167.99, 167.95, 156.55, 137.60, 136.27, 136.03, 135.49, 135.09, 134.91, 128.51, 128.47, 128.43, 128.39, 128.26, 128.22, 128.10, 128.04, 127.98, 127.95, 127.83, 127.78, 127.73, 77.20, 75.06, 74.99, 72.10, 69.58, 67.62, 67.58, 67.25, 66.41, 64.78, 62.24, 59.95, 34.06, 31.90, 29.68, 29.64, 29.60, 29.46, 29.34, 29.26, 29.12, 24.83, 22.67, 14.11. ^{31}P NMR ($CDCl_3$): δ −0.51, −0.55, −0.65, −0.71. HRMS (ESI, $[M + Na]^+$): Calcd for $C_{54}H_{74}NNaO_{11}P$: 966.4897. Found: 966.4891.

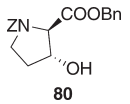
Compound 14.



14

Following the general procedure L, **79** (27.6 mg, 0.0292 mmol), Pd−C (3.0 mg), and MeOH-AcOH (5 mL) were used. The crude mixture was chromatographed ($CHCl_3$/MeOH/AcOH 9:0:1 to 8:1:1 to 7:2:1) to yield **14** (3.2 mg, 0.0059 mmol, 20%, white powder). ^{1}H NMR ($CDCl_3$: CD_3CO_2D = 4:1): δ 4.40−3.85 (6H, m), 3.65 (1H, m), 3.34 (1H, m), 2.95−2.70 (3H, m), 2.25 (2H, m), 1.52 (2H, m), 1.17 (28H, m), 0.78 (3H, t, J = 6.8 Hz). HRMS (ESI, $[M - H]^-$): Calcd for $C_{25}H_{49}NO_9P$: 538.3145. Found: 538.3108. mp 156.5−157.0 °C.

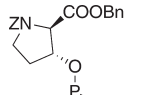
Synthesis of Compound 15−17 (Scheme 6). Synthesis of Compound 15. Compound 80.



80

Following the general procedure E, *trans*-3-hydroxy-L-proline (265.8 mg, 2.03 mmol), benzyl chloroformate (409.0 mg, 2.40 mmol), saturated aqueous $NaHCO_3$ (10 mL), and THF (10 mL) were used. The crude mixture was chromatographed ($CHCl_3$/MeOH 9:1 to 8:2) to yield *N*-Z-*trans*-3-hydroxy-L-proline (594.4 mg, 2.24 mmol, quantitative yield, colorless oil). The following benzylation was carried out in accordance with the general procedure F by using the intermediate alcohol (541.2 mg, 2.040 mmol), benzyl bromide (523.4 mg, 3.06 mmol), triethylamine (1.1 mL, 6.53 mmol), and THF (10 mL). The crude mixture was chromatographed (EtOAc/hexane 2:3) to yield **80** (213.0 mg, 0.620 mmol, quantitative yield, colorless oil). ^{1}H NMR ($CDCl_3$): δ 7.32 (10H, m), 5.15 (2H, m), 5.04 (2H, m), 4.43 (1H, m), 4.40−4.31 (1H, m), 3.68 (2H, m), 2.08 (1H, m), 1.92 (1H, m). HRMS (ESI, $[M + Na]^+$): Calcd for $C_{20}H_{21}NNaO_5$: 378.1317. Found: 378.1317.

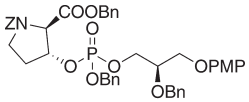
Compound 81.



81

Following the general procedure A, **80** (217.5 mg, 0.612 mmol), **27** (200.0 mg, 0.612 mmol), 1*H*-tetrazole (42.1 mg, 0.600 mmol), CH_2Cl_2 (5 mL), and THF (1.2 mL) were used. The crude mixture was chromatographed (EtOAc/hexane/Et_3N 100:0:3 to 20:80:3) to yield **81** (191.8 mg, 0.324 mmol, 54%, colorless oil). ^{1}H NMR (CD_2Cl_2): δ 7.32 (15H, m), 5.16−5.03 (4H, m), 4.95 (1H, m), 4.67 (2H, m), 4.51 (2H, m), 3.67 (1H, m), 3.60 (2H, m), 2.03 (2H, m), 1.20 (12H, m).

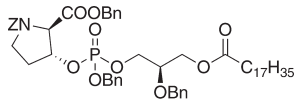
Compound 82.



82

Following the general procedure B, **43** (108.1 mg, 0.357 mmol), **81** (176.0 mg, 0.297 mmol), 1*H*-tetrazole (62.4 mg, 0.891 mmol), TBHP (0.24 mL, 1.44 mmol), CH_2Cl_2 (2 mL), and THF (1.5 mL) were used. The crude mixture was chromatographed (EtOAc/hexane 1:2) to yield **82** (96.1 mg, 0.121 mmol, 41%, colorless oil). ^{1}H NMR ($CDCl_3$): δ 7.27 (20H, m), 6.76 (4H, m), 5.12 (4H, m), 5.06−4.94 (5H, m), 4.62 (3H, m), 4.26 (1H, m), 4.11 (1H, m), 3.92 (2H, m), 3.84 (1H, m), 3.73 (3H, s), 3.66 (1H, m), 3.52 (1H, m), 2.46 (1H, m), 2.25 (1H, m). HRMS (ESI, $[M + Na]^+$): Calcd for $C_{44}H_{46}NNaO_{11}P$: 818.2706. Found: 818.2705.

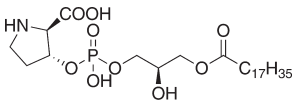
Compound 83.



83

Following the general procedure K, **82** (85.3 mg, 0.107 mmol), CAN (146.9 mg, 0.268 mmol), and CH_3CN-H_2O (2 mL) were used. The crude mixture was chromatographed (EtOAc/hexane 1:1 to 4:1) to yield the intermediate alcohol (45.8 mg, 0.0664 mmol, 62%, brown oil). The obtained alcohol (45.8 mg, 0.0664 mmol) was acylated with stearoyl chloride (24.2 mg, 0.0797 mmol) in CH_2Cl_2 (5 mL) in the presence of DMAP (16.2 mg, 0.133 mmol). The crude mixture was chromatographed (EtOAc/hexane 1:3) to yield **83** (59.3 mg, 0.0620 mmol, 93%, colorless oil). ^{1}H NMR ($CDCl_3$): δ 7.25 (20H, m), 5.14 (2H, m), 5.02 (2H, m), 4.93 (1H, m), 4.55 (3H, m), 4.17 (1H, m), 4.06 (3H, m), 3.69 (2H, m), 3,52 (1H, m), 2.25 (2H, m), 2.05 (2H, m), 1.55 (2H, m), 1.23 (28H, m), 0.86 (3H, t, J = 6.8 Hz). ^{13}C NMR ($CDCl_3$): δ 173.38, 169.11, 168.98, 154.05, 137.52, 136.34, 136.21, 135.15, 128.72, 128.60, 128.47, 128.40, 128.17, 128.06, 128.01, 127.97, 127.88, 127.78, 80.16, 79.17, 74.91, 72.10, 69.81, 69.76, 67.41, 67.28, 66.17, 66.52, 66.05, 62.14, 44.52, 44.19, 34.05, 31.90, 29.67, 29.63, 29.60, 29.45, 29.34, 29.26, 29.12, 24.83, 22.67, 14.11. ^{31}P NMR ($CDCl_3$): δ −1.41, −1.45, −1.54. HRMS (ESI, $[M + Na]^+$): Calcd for $C_{55}H_{74}NNaO_{11}P$: 978.4897. Found: 978.4890.

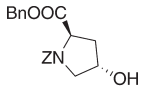
Compound 15.



15

Following the general procedure L, **83** (30.2 mg, 0.0316 mmol), Pd−C (3.0 mg), and MeOH-AcOH (5 mL) were used. The crude mixture was chromatographed ($CHCl_3$/MeOH/AcOH 9:0:1 to 8:1:1 to 7:2:1) to yield **15** (3.0 mg, 0.00540 mmol, 17%, white powder). ^{1}H NMR ($CDCl_3$/CD_3CO_2D 4:1): δ 5.22 (1H, m), 4.98 (1H, m), 4.09 (5H, m), 3.63−3.34 (3H, m), 3.08 (1H, m), 2.25 (2H, m), 1.53 (2H, m), 1.17 (28H, m), 0.78 (3H, t, J = 6.8 Hz). HRMS (ESI, [M − H]$^-$): Calcd for $C_{26}H_{49}NO_9P$: 550.3145. Found: 550.3111. mp 150.5−151.0 °C.

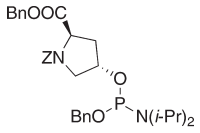
Synthesis of Compound 16. Compound 84.



84

Following the general procedure E, *trans*-4-hydroxy-L-proline (1.40 g, 10.7 mmol), benzyl chloroformate (2.05 g, 20.9 mmol), saturated aqueous $NaHCO_3$ (30 mL), and THF (60 mL) were used. The crude mixture was chromatographed ($CHCl_3$/MeOH 1:0 to 9:1) to yield *N*-Z-*trans*-4-hydroxy-L-proline (3.41 g, 12.9 mmol, quantitative yield, colorless oil). The following benzylation was carried out in accordance with the general procedure F by using the intermediate alcohol (789.5 mg, 2.977 mmol), benzyl bromide (619.2 mg, 3.572 mmol), triethylamine (2 mL, 14.67 mmol), and THF (10 mL). The crude mixture was chromatographed (EtOAc/hexane 1:2 to 2:1) to yield **84** (768.3 mg, 2.162 mmol, 94% (2 steps), colorless oil). ^{1}H NMR ($CDCl_3$): δ 7.32 (10H, m), 5.14 (2H, m), 5.03 (2H, m), 4.56 (1H, m), 4.49 (1H, m), 3.70−3.50 (2H, m), 2.29 (1H, m), 2.08 (1H, m). HRMS (ESI, [M + Na]$^+$): Calcd for $C_{20}H_{21}NNaO_5$: 378.1317. Found: 378.1319.

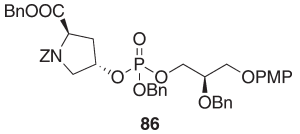
Compound 85.



85

Following the general procedure A, **84** (204.9 mg, 0.577 mmol), **27** (174.7 mg, 0.577 mmol), 1*H*-tetrazole (40.4 mg, 0.577 mmol), CH_2Cl_2 (5 mL), and THF (1.2 mL) were used. The crude mixture was chromatographed (EtOAc/hexane/Et_3N 100:0:3 to 20:80:3) to yield **85** (167.0 mg, 0.282 mmol, 49%, colorless oil). ^{1}H NMR (CD_2Cl_2): δ 7.32 (15H, m), 5.15−4.45 (8H, m), 3.66 (2H, m), 3.52 (1H, m), 3.23 (1H, m), 2.40 (1H, m), 2.09 (2H, m), 1.20 (12H, m).

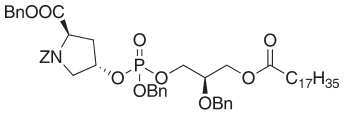
Compound 86.



86

Following the general procedure B, **43** (89.3 mg, 0.295 mmol), **85** (145.5 mg, 0.246 mmol), 1*H*-tetrazole (51.7 mg, 0.738 mmol), TBHP (0.12 mL, 0.72 mmol), CH_2Cl_2 (2 mL), and THF (1.5 mL) were used. The crude mixture was chromatographed (EtOAc/hexane 1:2) to yield **86** (70.7 mg, 0.089 mmol, 36%, colorless oil). ^{1}H NMR ($CDCl_3$): δ 7.30 (20H, m), 6.76 (4H, m), 5.20−4.94 (6H, m), 4.87 (1H, m), 4.64 (2H, m), 4.42 (1H, m) 4.24 (1H, m), 4.13 (1H, m), 3.92 (2H, m), 3.87 (1H, m), 3.78−3.67 (4H, m), 3.56 (1H, m), 2.41 (1H, m), 1.98 (1H, m). HRMS (ESI, [M + Na]$^+$): Calcd for $C_{44}H_{46}NNaO_{11}P$: 818.2706. Found: 818.2707.

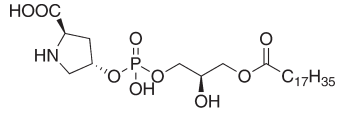
Compound 87.



87

Following the general procedure K, **86** (88.4 mg, 0.111 mmol), CAN (152.2 mg, 0.278 mmol), and CH_3CN-H_2O (2 mL) were used. The crude mixture was chromatographed (EtOAc/hexane 1:1 to 4:1) to yield the intermediate alcohol. The obtained alcohol was acylated with stearoyl chloride (43.0 mg, 0.142 mmol) in CH_2Cl_2 (5 mL) in the presence of DMAP (40.7 mg, 0.333 mmol). The crude mixture was chromatographed (EtOAc/hexane 1:3) to yield **87** (62.2 mg, 0.0650 mmol, 59% (2 steps), colorless oil). ^{1}H NMR ($CDCl_3$): δ 7.29 (20H, m), 5.20−4.94 (6H, m), 4.88 (1H, m), 4.57 (2H, m), 4.44 (1H, m), 4.18 (1H, m), 4.08 (3H, m), 3.65 (2H, m), 3,58 (1H, m), 2.42 (1H, m), 2.25 (2H, m), 2.01 (1H, m), 1.55 (2H, m), 1.23 (28H, m), 0.86 (3H, t, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$): δ 173.39, 171.87, 171.65, 154.57, 153.99, 137.53, 136.25, 136.11, 135.39, 135.17, 128.74, 128.64, 128.55, 128.47, 128.41, 128.31, 128.17, 128.11, 128.07, 128.01, 127.90, 127.86, 127.84, 127.80, 127.78, 77.20, 76.28, 75.76, 75.71, 75.64, 75.59, 75.71, 75.64, 75.59, 75.09, 75.04, 74.96, 72.13, 69.76, 69.70, 67.34, 67.07, 66.96, 66.93, 66.46, 66.41, 62.14, 57.69, 57.42, 57.38, 53.60, 53.53, 53.46, 53.04, 37.84, 37.79, 36.78, 34.06, 31.89, 29.67, 29.63, 29.59, 29.45, 29.33, 29.25, 29.11, 24.83, 22.66, 14.09. ^{31}P NMR ($CDCl_3$): δ −1.20. HRMS (ESI, [M + Na]$^+$) Calcd for $C_{55}H_{74}NNaO_{11}P$: 978.4897. Found: 978.4920.

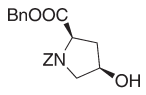
Compound 16.



16

Following the general procedure L, **87** (31.5 mg, 0.0329 mmol), Pd−C (3.0 mg), and MeOH-AcOH (5 mL) were used. The crude mixture was chromatographed ($CHCl_3$/MeOH/AcOH 9:0:1 to 8:1:1 to 7:2:1) to yield **16** (12.7 mg, 0.0230 mmol, 70%, white powder). ^{1}H NMR ($CDCl_3$/CD_3CO_2D 4:1): δ 5.06 (1H, m), 4.67−4.37 (1H, m), 4.05 (2H, m), 3.97−3.80 (3H, m), 3.48 (2H, m), 3.04 (1H, m), 2.70 (1H, m), 2.25 (2H, m), 1.52(2H, m), 1.17 (28H, m), 0.78 (3H, t, J = 6.8 Hz). HRMS (ESI, [M − H]$^-$): Calcd for $C_{26}H_{49}NO_9P$: 550.3145. Found: 550.3135. mp 188.0−188.5 °C.

Synthesis of Compound 17. Compound 88.

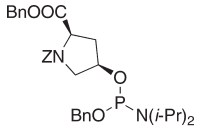


88

Following the general procedure E, *cis*-4-hydroxy-L-proline (164.7 mg, 1.26 mmol), benzyl chloroformate (257.1 mg, 1.51 mmol), satd $NaHCO_3$ (5 mL), and THF (5 mL) were used. The crude mixture was chromatographed ($CHCl_3$/MeOH 1:0 to 9:1) to yield *N*-Z-*cis*-4-hydroxy-L-proline (316.1 mg, 1.19 mmol, 95%, colorless oil). The

following benzylation was carried out in accordance with the general procedure F by using the intermediate alcohol (316.1 mg, 1.19 mmol), benzyl bromide (305.6 mg, 1.79 mmol), triethylamine (0.60 mL, 3.58 mmol), and THF (10 mL). The crude mixture was chromatographed (EtOAc/hexane 2:3) to yield **88** (321.5 mg, 0.905 mmol, 72% (2 steps), colorless oil). ^{1}H NMR ($CDCl_3$): δ 7.32 (10H, m), 5.28−4.99 (6H, m), 4.66 (1H, m), 4.66 (1H, m), 3.73 (1H, m), 3.63 (1H, m), 2.34 (2H, m), 2.11 (2H, m). HRMS (ESI, [M + Na]$^+$): Calcd for $C_{20}H_{21}NNaO_5$: 378.1317. Found: 378.1319.

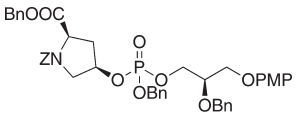
Compound 89.



89

Following the general procedure A, **88** (217.5 mg, 0.612 mmol), **27** (198.0 mg, 0.600 mmol), 1*H*-tetrazole (42.1 mg, 0.600 mmol), CH_2Cl_2 (5 mL), and THF (1.2 mL) were used. The crude mixture was chromatographed (EtOAc/hexane/Et_3N 100:0:3 to 20:80:3) to yield **89** (235.5 mg, 0.398 mmol, 66%, colorless oil). ^{1}H NMR (CD_2Cl_2): δ 7.32 (15H, m), 5.10 (5H, m), 4.64 (2H, m), 4.48 (2H, m), 3.73 (1H, m), 3.58 (2H, m), 2.38 (2H, m), 1.20 (12H, m).

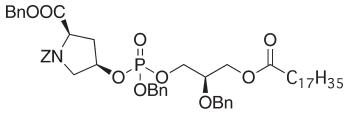
Compound 90.



90

Following the general procedure B, **43** (132.3 mg, 0.437 mmol), **89** (215.4 mg, 0.364 mmol), 1*H*-tetrazole (76.5 mg, 1.092 mmol), TBHP (0.18 mL, 1.08 mmol), CH_2Cl_2 (2 mL), and THF (2 mL) were used. The crude mixture was chromatographed (EtOAc/hexane 2:3 to 1:1) to yield **90** (169.1 mg, 0.212 mmol, 58%, colorless oil). ^{1}H NMR ($CDCl_3$): δ 7.30 (20H, m), 6.77 (4H, m), 5.17−4.89 (7H, m), 4.64 (2H, m), 4.53−4.42 (1H, m), 4.22 (1H, m), 4.10 (1H, m), 3.93 (2H, m), 3.85 (1H, m), 3.73−3.57 (5H, m), 2.41 (1H, m), 2.27 (1H, m). HRMS (ESI, [M + Na]$^+$): Calcd for $C_{44}H_{46}NNaO_{11}P$: 818.2706. Found: 818.2705.

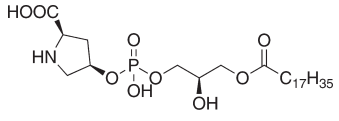
Compound 91.



91

Following the general procedure K, **90** (116.7 mg, 0.147 mmol), CAN (201.0 mg, 0.367 mmol), and CH_3CN-H_2O (2 mL) were used. The crude mixture was chromatographed (EtOAc/hexane 1:1 to 4:1) to yield the intermediate alcohol (90.8 mg, 0.132 mmol, 90%, brown oil). The alcohol (90.8 mg, 0.132 mmol) was acylated with stearoyl chloride (47.9 mg, 0.158 mmol) in CH_2Cl_2 (5 mL) in the presence of DMAP (32.2 mg, 0.263 mmol). The crude mixture was chromatographed (EtOAc/hexane 1:2) to yield **91** (92.7 mg, 0.097 mmol, 73%, colorless oil). ^{1}H NMR ($CDCl_3$): δ 7.30 (20H, m), 5.18−4.87 (7H, m), 4.57 (2H, m), 4.42 (1H, m), 4.17 (1H, m), 4.08 (2H, m), 3.95 (1H, m), 3.73 (3H, m), 2.38 (2H, m), 2.25 (2H, m), 1.55 (2H, m), 1.23 (28H, m), 0.86 (3H, t, J = 6.8 Hz). ^{13}C NMR ($CDCl_3$): δ 173.37, 173.08, 171.01, 171.08, 170.75, 154.49, 154.06, 137.63, 137.60, 137.56, 136.26, 136.21, 135.50, 135.43, 135.38, 128.66, 128.61, 128.55, 128.51, 128.46, 128.38, 128.21, 128.13, 128.07, 128.01, 127.97, 127.94, 127.86, 127.83, 127.81, 127.74, 77.21, 76.31, 76.26, 76.21, 75.40, 75.35, 75.31, 75.12, 75.05, 72.12, 72.07, 69.58, 69.53, 69.48, 67.32, 67.29, 67.23, 66.96, 66.88, 66.46, 66.40, 66.35, 66.29, 65.81, 62.23, 57.69, 57.44, 53.49, 53.44, 53.39, 53.11, 53.05, 53.00, 37.62, 37.58, 36.67, 36.61, 34.05, 31.88, 30.06, 29.66, 29.62, 29.59, 29.44, 29.32, 29.25, 29.11, 24.83, 15.24, 14.09. ^{31}P NMR ($CDCl_3$): δ −1.51, −1.58, −1.65. HRMS (ESI, [M + Na]$^+$): Calcd for $C_{55}H_{74}NNaO_{11}P$: 978.4897. Found: 978.4897.

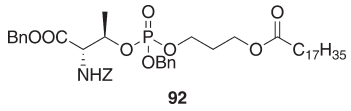
Compound 17.



17

Following the general procedure L, **91** (32.2 mg, 0.0336 mmol), Pd−C (3.0 mg), and MeOH-AcOH (5 mL) were used. The crude mixture was chromatographed ($CHCl_3$/MeOH/AcOH 9:0:1 to 8:1:1 to 7:2:1) to yield **17** (13.1 mg, 0.0237 mmol, 71%, white powder). ^{1}H NMR ($CDCl_3$/CD_3CO_2D 4:1): δ 5.03 (1H, m), 4.64 (1H, m), 4.23 (1H, m), 4.05 (2H, m), 3.96 (2H, m), 3.87 (2H, m), 3.32 (1H, m), 3.00 (1H, m), 2.55 (1H, m), 2.24 (2H, m), 1.52(2H, m), 1.16 (28H, m), 0.78 (3H, t, J = 6.8 Hz). HRMS (ESI, [M − H]$^-$): Calcd for $C_{26}H_{49}NO_9P$: 550.3145. Found: 550.3096. mp 198.5−199.0 °C.

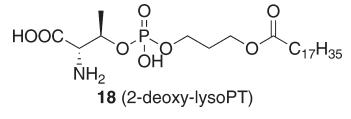
Synthesis of 2-Deoxy-lysoPT Derivatives 18 and 19 (Scheme 7). Synthesis of Compound 18. Compound 92.



92

Following the general procedure B, **24** (125.5 mg, 0.366 mmol), **65** (175.2 mg, 0.302 mmol), 1*H*-tetrazole (63.1 mg, 0.900 mmol), TBHP (0.15 mL, 0.900 mmol), CH_2Cl_2 (2 mL), and THF (2 mL) were used. The crude mixture was chromatographed (EtOAc/hexane 1:3) to yield **92** (193.1 mg, 0.230 mmol, 76%, colorless oil). ^{1}H NMR ($CDCl_3$): δ 7.31 (15H, m), 5.56 (1H, m), 5.22−4.87 (7H, m), 4.46 (1H, m), 4.08 (2H, m), 3.98 (2H, m), 2.23 (2H, m), 1.86 (2H, m), 1.55 (2H, m), 1.38 ($^3/_2$H, d, J = 6.4 Hz), 1.33 ($^3/_2$H, d, J = 6.4 Hz), 1.23 (28H, m), 0.86 (3H, t, J = 6.8 Hz). ^{13}C NMR ($CDCl_3$): δ 173.55, 175.51, 169.25, 156.41, 135.92, 135.52, 135.44, 134.85, 134.83, 128.63, 128.52, 128.48, 128.41, 128.38, 128.34, 128.29, 128.23, 128.11, 127.96, 127.90, 127.76, 75.24, 69.31, 67.58, 67.16, 65.70, 64.45, 59.95, 58.46, 58.40, 34.03, 31.79, 29.57, 29.53, 29.49, 29.35, 29.30, 29.23, 29.14, 29.02, 24.76, 22.57, 18.40, 18.31, 15,15, 14.10. ^{31}P NMR ($CDCl_3$): δ −1.26, −1.35. HRMS (ESI, [M + Na]$^+$): Calcd for $C_{47}H_{68}NNaO_{10}P$: 860.4479. Found: 860.4469.

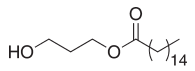
Compound 18 (2-Deoxy-lysoPT).



18 (2-deoxy-lysoPT)

Following the general procedure L, **92** (47.6 mg, 0.0568 mmol), Pd−C (3.0 mg), and MeOH-AcOH (5 mL) were used. The crude mixture was chromatographed ($CHCl_3$/MeOH/AcOH 9:0:1 to 8:1:1) and the product was washed with MeOH to yield **18** (8.6 mg, 0.0165 mmol, 29%, white powder). ^{1}H NMR ($CDCl_3$/CD_3CO_2D 4:1): δ 4.88 (1H, m), 4.09 (3H, m), 3.99 (2H, m), 2.22 (2H, m), 1.90 (2H, m), 1.50 (2H, m), 1.17 (31H, m), 0.78 (3H, t, J = 6.6 Hz). HRMS (ESI, [M − H]$^-$): Calcd for $C_{25}H_{50}NO_8P$ + $^4/_3CF_3CO_2H$: C, 49.18; H, 7.66; N, 2.07. Found: C, 49.24; H, 7.99; N, 1.86. mp 177.5−178.0 °C.

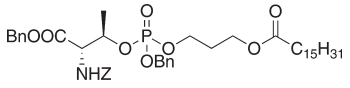
Synthesis of Compound 19. Compound 93.



93

To a solution of 1,3-propane diol (3.066 g, 40.3 mmol) and pyridine (1.0 mL, 12 mmol) in CH_2Cl_2 (8 mL), a solution of palmitoyl chloride (2.744 g, 9.99 mmol) in CH_2Cl_2 (8 mL) was added dropwise and the whole was stirred for 18 h at room temperature. After quenching with 3N aqueous HCl, the separated organic layer was washed with 3N aqueous HCl and brine and the organic layer was dried over Na_2SO_4. The organic solvent was evaporated, and the residue was chromatographed (EtOAc: hexane = 1: 3) to yield **93** (1.394 g, 4.44 mmol, 44%, colorelss solid). ^{1}H NMR ($CDCl_3$): δ 4.22 (2H, t, J = 6.1 Hz), 3.67 (2H, m), 2.29 (2H, t, J = 7.6 Hz), 1.85 (2H, m), 1.59 (2H, m), 1.23 (24H, m), 0.86 (3H, t, J = 6.8 Hz).

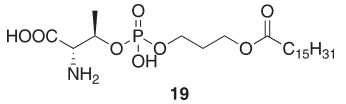
Compound 94.



94

Following the general procedure C, **93** (360.0 mg, 1.15 mmol), **65** (585.7 mg, 1.01 mmol), 1*H*-tetrazole (192.5 mg, 2.75 mmol), TBHP (0.45 mL, 2.70 mmol), CH_2Cl_2 (6 mL), and THF (6 mL) were used. The crude mixture was chromatographed (EtOAc: hexane = 1: 2) to give **94** (502.5 mg, 0.621 mmol, 61%, colorless oil). ^{1}H NMR ($CDCl_3$): δ 7.31 (15H, m), 5.57 (1H, m), 5.22−4.90 (7H, m), 4.47 (1H, m), 4.09 (2H, m), 3.97 (2H, m), 2.23 (2H, t, J = 7.5 Hz), 1.87 (2H, m), 1.55 (2H, m), 1.39 (3/2H, d, J = 6.4 Hz), 1.33 (3/2H, d, J = 6.4 Hz), 1.23 (24H, m), 0.86 (3H, t, J = 6.8 Hz).

Compound 19.



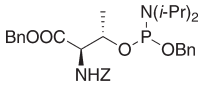
19

A solution of **94** (223.5 mg, 0.276 mmol) in MeOH (16 mL) and AcOH (4 mL) was hydrogenataed with Pd−C (20.0 mg) for 1 day at rt. After filtration, the solvent was evaporated and the residue was chromatographed ($CHCl_3$:MeOH:AcOH = 9:0:1 → 8:1:1) to yield **19** (44.0 mg, 0.0888 mmol, 32%, colorless powder). ^{1}H NMR ($CDCl_3$:CD_3COOD = 4:1): δ 4.83 (1H, m), 4.08 (2H, m), 3.86 (3H, m), 2.21 (2H, m), 1.86 (2H, m), 1.52 (2H, m), 1.33 (3H, m), 1.18 (24H, m), 0.79 (3H, t, J = 6.8 Hz). HRMS (ESI, [M − H]$^-$): Calcd for $C_{23}H_{45}NO_8P$: 494.2883. Found: 494.2863.

Synthesis of Compound 19-D. *N*-Z-D-Threonine. D-Threonine (2.027 g, 17.0 mmol) was dissolved in saturated aqueous $NaHCO_3$ (100 mL) and THF (50 mL) and the whole was cooled to 0 °C. To this solution, a solution of benzyl chloroformate (3.481 g, 20.4 mmol) in THF (50 mL) was added at 0 °C and the whole was stirred for 1.5 h at 0 °C. After quenching with 3N aqueous HCl, the separated organic layer was washed with 3N aqueous HCl and brine and dried over Na_2SO_4. The organic solvent was evaporated, and the residue was chromatographed ($CHCl_3$:MeOH = 1:0 → 9:1) to give N-Z-D-threonine (3.671 g, 85%, pale-yellow oil). ^{1}H NMR ($CDCl_3$): δ 7.30 (m, 5H), 5.93 (1H, d, J = 8.8 Hz), 4.37 (1H, m), 4.31 (1H, d, J = 8.6 Hz), 1.18 (3H, d, J = 6.2 Hz).

***N*-Z-D-Threonine Benzyl Ester.** Following the general procedure F, *N*-Z-D-threonine (3.661 g, 14.5 mmol), TBAI (1.041 g, 2.00 mmol), triethylamine (5.80 mL), benzyl bromide (2.10 mL), and THF (71 mL) were used. The crude mixture was chromatographed (EtOAc:hexane = 1:1) to yield *N*-Z-D-threonine benzyl ester (2.51 g, 7.31 mmol, 50%, white solid). ^{1}H NMR ($CDCl_3$): δ 7.33 (10H, m), 5.52 (1H, m), 5.19 (2H, m), 5.11 (2H, m), 4.36 (2H, m), 1.76 (1H, brs), 1.22 (3H, d, J = 6.4 Hz).

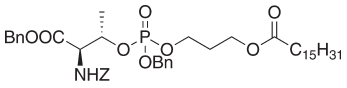
Compound 65-D.



65-D

Following the general procedure B, **27** (2.032 g, 6.00 mmol), *N*-Z-D-threonine benzyl ester (2.060 g, 6.00 mmol), 1*H*-tetrazole (420 mg, 6.00 mmol), CH_2Cl_2 (48 mL), and THF (12 mL) were used. The mixture was chromatographed (hexane:EtOAc:Et_3-N = 35:4:1) to yield **65-D** (2.785 g, 4.80 mmol, 80%, colorless oil). ^{1}H NMR (CD_2Cl_2): δ 7.34 (15H, m), 5.61 (1H, d, J = 9.3 Hz), 5.51 (1H, d, J = 9.3 Hz), 5.12 (4H, m), 4.71 (1H, m), 4.60 (2H, m), 4.36 (1H, m), 3.60 (2H, m), 1.31 (3H, m), 1.17 (12H, m).

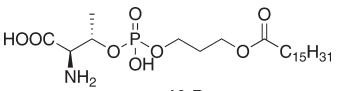
Compound 94-D.



94-D

Following the general procedure C, **93** (158.7 mg, 0.505 mmol), **65-D** (303.3 mg, 0.522 mmol), 1*H*-tetrazole (70.0 mg, 1.00 mmol), TBHP (0.24 mL, 1.44 mmol), CH_2Cl_2 (3 mL), and THF (3 mL) were used. The crude mixture was chromatographed (EtOAc: hexane = 1:2) to give **94-D** (336.3 mg, 0.416 mmol, 82%, colorless oil). ^{1}H NMR ($CDCl_3$): δ 7.31 (15H, m), 5.57 (1H, m), 5.22−4.91 (7H, m), 4.47 (1H, m), 4.09 (2H, m), 3.97 (2H, m), 2.23 (2H, m), 1.86 (2H, m), 1.55 (2H, m), 1.39 (3H, d, J = 6.4 Hz), 1.33 (3H, d, J = 6.4 Hz), 1.25 (24H, m), 0.86 (3H, t, J = 6.8 Hz).

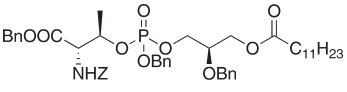
Compound 19-D.



19-D

Following the general procedure D, **94-D** (98.4 mg, 0.122 mmol), Pd−C (5.0 mg), and MeOH-AcOH (10 mL) were used. The crude mixture was chromatographed ($CHCl_3$:MeOH: AcOH = 6:3:1), and the product was precipitated by using MeOH to yield **19-D** (17.9 mg, 0.0361 mmol 30%). ^{1}H NMR ($CDCl_3$:CD_3CO_2D = 4:1): δ 4.76 (1H, m), 4.08 (2H, m), 3.84 (3H, m), 2.21 (2H, m), 1.84 (2H, m), 1.52 (2H, m), 1.34 (3H, m), 1.18 (24H, m), 0.79 (3H, t, J = 6.8 Hz). HRMS (ESI, [M − H]$^-$): Calcd for $C_{23}H_{45}NO_8P$: 494.2883. Found: 494.2912.

Synthesis of LysoPT Analogues 20 (C-12) and 21 (C-16) (Scheme 8). Synthesis of Compound 20 (LysoPT C-12). Compound 95.

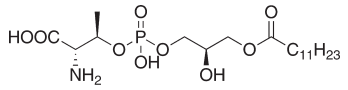


95

Following the general procedure K, **67** (250.1 mg, 0.319 mmol), CAN (441.4 mg, 0.805 mmol), and CH_3CN-H_2O (3 mL) were used. The crude mixture was chromatographed (EtOAc/hexane 1:1 to 4:1) to yield the intermediate alcohol (154.6 mg, 0.228 mmol, 71%, brown oil). The obtained alcohol (219.1 mg, 0.323 mmol) was acylated with lauroyl chloride (84.7 mg, 0.387 mmol) in CH_2Cl_2 (10 mL) in the presence of DMAP (118.4 mg, 0.969 mmol). The crude mixture was

chromatographed (EtOAc/hexane 1:3 to 2:3) to yield **95** (154.6 mg, 0.180 mmol, 56%, colorless oil). ^{1}H NMR ($CDCl_3$): δ 7.31 (20H, m), 5.57 (1H, m), 5.20−4.89 (7H, m), 4.56 (2H, m), 4.48 (1H, m), 4.19 (1H, m), 4.08−3.96 (3H, m), 3.70 (1H, m), 2.26, (2H, m), 1.55 (2H, m), 1.36 ($^3/_2$H, d, J=6.4 Hz), 1.31 ($^3/_2$H, d, J=6.4 Hz), 1.23 (16H, m), 0.85 (3H, t, J=7.0 Hz). ^{13}C NMR ($CDCl_3$): δ 173.28, 169.24, 169.21, 156.39, 137.50, 137.46, 135.93, 135.91, 135.39, 134.86, 134.82, 128.51, 128.46, 128.40, 128.33, 128.27, 128.23, 128.10, 127.95, 127.81, 127.76, 127.73, 127.70, 127.66, 75.42, 75.37, 75.32, 74.92, 74.84, 74.76, 71.98, 71.87, 69.43, 69.37, 69.33, 67.60, 67.56, 67.17, 67.15, 66.24, 66.18, 66.12, 65.69, 62.12, 58.44, 58.38, 33.93, 31.76, 29.46, 29.32, 29.19, 29.13, 28.98, 24.71, 22.54, 18.39, 18.32, 15.15, 14.00. ^{31}P NMR ($CDCl_3$): δ −1.32, −1.38. HRMS (ESI, [M + Na]$^+$): Calcd for $C_{48}H_{62}NNaO_{11}P$: 882.3958. Found: 882.3957.

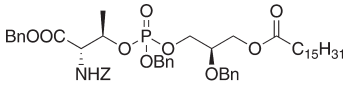
Compound 20 (LysoPT-C12).



20 (lysoPT-C12)

Following the general procedure L, **95** (46.5 mg, 0.0541 mmol), Pd−C (3.0 mg), and MeOH-AcOH (5 mL) were used. The crude mixture was chromatographed ($CHCl_3$/MeOH/AcOH 9:0:1 to 8:1:1 to 7:2:1) and the product was washed with MeOH to yield **20** (15.8 mg, 0.0347 mmol, 64%, white powder). ^{1}H NMR ($CDCl_3$/CD_3CO_2D 4:1): δ 4.90 (1H, m), 4.16−3.99 (6H, m), 2.25 (2H, m), 1.49(2H, m), 1.17 (19H, m), 0.78 (3H, t, J = 6.8 Hz). HRMS (ESI, [M − H]$^-$): Calcd for $C_{19}H_{37}NO_9P$: 454.2206. Found: 454.2168. mp 164.5−165.0 °C.

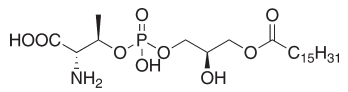
Synthesis of Compound 21 (LysoPT C-16). Compound 96.



96

Following the general procedure K, **67** (250.1 mg, 0.319 mmol), CAN (441.4 mg, 0.805 mmol), and CH_3CN-H_2O (3 mL) were used. The crude mixture was chromatographed (EtOAc/hexane 1:1 to 4:1) to yield the intermediate alcohol (154.6 mg, 0.228 mmol, 71%, brown oil). The obtained alcohol (219.1 mg, 0.323 mmol) was acylated with palmitoyl chloride (106.4 mg, 0.387 mmol) in CH_2Cl_2 (10 mL) in the presence of DMAP (118.4 mg, 0.969 mmol). The crude mixture was chromatographed (EtOAc/hexane 1:3 to 2:3) to yield **96** (146.7 mg, 0.160 mmol, 50%, colorless oil). ^{1}H NMR ($CDCl_3$): δ 7.31 (20H, m), 5.56 (1H, m), 5.21−4.87 (7H, m), 4.57 (1H, m), 4.46 (1H, m), 4.19 (1H, m), 4.08−3.94 (3H, m), 2.24 (3H, m), 1.55 (2H, m), 1.36 ($^3/_2$H, d, J=6.4 Hz), 1.31 ($^3/_2$H, d, J=6.4 Hz), 1.26 (25H, m), 0.85 (3H, t, J= 7.0 Hz). ^{13}C NMR ($CDCl_3$): δ 173.42, 169.32, 156.48, 137.59, 137.55, 135.98, 135.48, 134.95, 134.91, 128.62, 128.57, 128.52, 128.45, 128.38, 128.22, 128.07, 127.91, 127.86, 127.82, 127.76, 77.20, 75.48, 75.02, 74.94, 74.86, 72.11, 72.00, 69.54, 69.49, 69.44, 67.72, 67.69, 67.30, 66.29, 62.24, 58.55, 58.47, 34.05, 31.89, 29.66, 29.62, 29.59, 29.44, 29.33, 29.25, 29.10, 24.81, 22.66, 18.51, 18.43, 15.24, 14.10. ^{31}P NMR ($CDCl_3$): δ −1.24, −1.30. HRMS (ESI, [M + Na]$^+$): Calcd for $C_{52}H_{70}NNaO_{11}P$: 938.4584. Found: 938.4595.

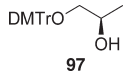
Compound 21.



21 (lysoPT-C16)

Following the general procedure L, **96** (44.1 mg, 0.0482 mmol), Pd−C (3.0 mg), and MeOH-AcOH (5 mL) were used. The crude mixture was chromatographed ($CHCl_3$/MeOH/AcOH 9:0:1 to 8:1:1 to 7:2:1) and the product was washed with MeOH to yield **21** (12.8 mg, 0.0250 mmol, 52%, white powder). ^{1}H NMR ($CDCl_3$/CD_3CO_2D 4:1): δ 4.80 (1H, m), 4.14−3.85 (6H, m), 2.24 (2H, m), 1.49 (2H, m), 1.17 (27H, m), 0.78 (3H, t, J = 6.8 Hz). HRMS (ESI, [M − H]$^-$): Calcd for $C_{23}H_{45}NO_9P$: 510.2832. Found: 510.2839. mp 173.0−173.5 °C.

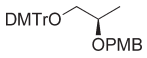
Synthesis of 22 (1-Deoxy-lysoPT) (Scheme 9). Compound 97.



97

Following the general procedure G, (*R*)-1,2-propanediol (1.20 g, 15.74 mmol), TBAI (1.627 mg, 4.41 mmol), DIPEA (3.93 g, 5.30 mL, 30.43 mmol), 4,4-dimethoxy tritylchloride (5.59 g, 16.50 mmol), and CH_2Cl_2 (100 mL) were used. The crude mixture was chromatographed (EtOAc:hexane=1:4→1:3) to yield **97** (5.98 g, 15.79 mmol, quantitative yield, yellow oil). ^{1}H NMR ($CDCl_3$): δ 7.45−7.19 (11H, m), 6.81 (4H, m), 3.96 (1H, m), 3.77 (3H, s), 3.61−3.37, 3.11−2.95 (3H, m), 1.15−1.08 (3H, m).

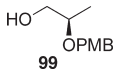
Compound 98.



98

To a solution of **97** (2.191 g, 5.79 mmol) and TBAI (641.4 mg, 1.736 mmol) in THF, NaH (55% in mineral oil, 631.4 mg, 14.47 mmol) was added at 0 °C and the whole was stirred for 10 min. To this mixture, *p*-methoxybenzyl chloride (1.09 g, 6.946 mmol) was added, and the whole was heated to 70 °C and stirred for 16 h at 70 °C. The reaction was quenched with ice water, and the organic layer was diluted with EtOAc, washed with brine, and dried over Na_2SO_4. The organic solvent was evaporated, chromatographed to yield **98** (2.235 g, 4.483 mmol, 78%, yellow oil). ^{1}H NMR ($CDCl_3$): δ 7.45−7.13 (13H, m), 6.87 (4H, m), 4.59−4.38 (2H, m), 3.78 (9H, m), 3.57−3.47, 3.20−2.97 (3H, m), 1.14 (3H, d, J = 6.0 Hz)

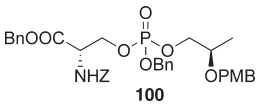
Compound 99.



99

To a solution of **98** (2.103 g, 4.218 mmol) in CH_2Cl_2-MeOH (1:3, 10 mL), PPTS (212.1 mg, 0.844 mmol) was added and the whole was stirred at rt for 1 h. The reaction was quenched with triethylamine, the solvent was evaporated, and the residue was chromatographed to yield **99** (726.5 mg, 3.702 mmol, 88%, colorless oil). ^{1}H NMR ($CDCl_3$): δ 7.26 (1H, m), 7.24 (1H, m), 6.88 (1H, m), 6.86 (1H, m), 4.57 (1H, m), 4.38 (1H, m), 3.79 (3H, s), 3.64 (1H, m), 3.57 (1H, m), 3.47 (1H, m), 1.14 (3H, d, J = 6.4 Hz).

Compound 100.

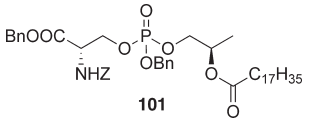


100

Following the general procedure B, **99** (196.2 mg, 1.00 mmol), **28** (225.9 mg, 0.399 mmol), 1*H*-tetrazole (84.1 mg, 1.20 mmol), TBHP (0.2 mL, 1.20 mmol), CH_2Cl_2 (2 mL), and THF (2.4 mL) were used. The crude mixture was chromatographed (EtOAc: hexane=1:2) to yield **100** (199.8 mg, 0.295 mmol, 74%, colorless oil). ^{1}H NMR ($CDCl_3$): δ 7.36−7.17 (15H, m), 6.80 (4H, m), 5.90 (1H, m), 5.18−5.08 (4H, m), 4.94 (2H, m), 4.56 (1H, m),

4.43 (2H, m), 4.39 (1H, m), 4.26 (1H, m), 3.90 (2H, m), 3.75 (3H, s), 3.63 (1H, m), 1.08 (3H, m).

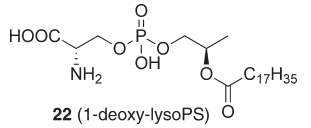
Compound 101.



101

To a solution of **100** (101.6 mg, 0.15 mmol) in CH_2Cl_2-phosphate buffer (1:1, 2 mL), DDQ (85.1 mg, 0.375 mmol) was added at 0 °C and the whole was stirred for 1 h. The reaction mixture was diluted with EtOAc, and the separated organic solvent was washed with saturated aqueous $NaHCO_3$ and brine and dried over Na_2SO_4. The solvent was evaporated, and the residue was chromatographed (EtOAc:hexane = 1:1) to yield the intermediate alcohol. To a solution of the obtained alcohol and DMAP (61.1 mg, 0.500 mg) in CH_2Cl_2, a solution of stearoyl chloride (54.5 mg, 0.18 mmol) in CH_2Cl_2 was added at 0 °C and the whole was stirred for 1 h. The solvent was evaporated and the residue was chromatographed (EtOAc:hexane = 1:3) to yield **101** (26.5 mg, 0.0326 mmol, 22%, colorless oil). ^{1}H NMR ($CDCl_3$): δ 7.31 (15H, m), 5.85 (1H, m), 5.16 (2H, m), 5.09 (2H, m), 4.96 (3/2H, m), 4.58 (3/2H, m), 4.42 (1H, m), 4.27 (1H, m), 4.08−3.84 (2H, m), 2.21 (2H, m), 1.57 (2H, m), 1.22−1.14 (31H, m), 0.86 (3H, t, J = 6.8 Hz). ^{13}C NMR ($CDCl_3$): δ 173.40, 168.78, 155.85, 136.07, 135.52, 134.96, 128.77, 128.64, 128.55, 128.31, 128.24, 128.12, 128.02, 127.98, 127.92, 127.88, 77.20, 73.71, 69.51, 67.77, 67.21, 66.49, 66.42, 54.54, 34.33, 33.99, 31.94, 29.72, 29.68, 29.64, 29.49, 29.38, 29.29, 29.14, 24.86, 24.79, 22.71, 18.05, 16.01, 14.14. ^{31}P NMR ($CDCl_3$): δ −0.94, −0.96, −1.71, −1.74. HRMS (ESI, [M + Na] $^+$): Calcd for $C_{46}H_{66}NNaO_{10}P^+$: 846.4322. Found: 846.4303.

Compound 22 (1-Deoxy-lysoPT).



22 (1-deoxy-lysoPS)

Following the general procedure L, **101** (29.4 mg, 0.0357 mmol), Pd−C (3.0 mg), and MeOH-AcOH (5 mL) were used. The crude mixture was chromatographed ($CHCl_3$:MeOH:AcOH = 9:0:1 → 8:1:1 → 7:2:1), and the product was precipitated by using MeOH to yield **22** (12.0 mg, 0.0235 mmol, 66%, white powder). ^{1}H NMR ($CDCl_3$:CD_3CO_2D = 4:1): δ 4.41 (3H, m), 4.20 (1H, m), 4.00 (1H, m), 3.82 (1H, m), 2.24 (2H, m), 1.53(2H, m), 1.17 (31H, m), 0.79 (3H, t, J = 6.6 Hz). HRMS (ESI, [M − H]$^-$): Calcd for $C_{24}H_{47}NO_8P^-$: 508.3039. Found: 508.3026. Anal. Calcd for $C_{24}H_{48}NO_8P \cdot {}^6/_7CF_3CO_2H$: C, 50.85; H, 8.11; N, 2.31. Found: C, 50.74; H, 8.44; N, 2.13. mp 152.5−153.0 °C.

Mast Cell Degranulation in Vitro. Mast cells from the peritoneal cavity of mice and rats were prepared essentially as described previously.[8] The mast cells were suspended at a cell density of 5×10^4/mL (in 0.2 mL) in HEPES-buffered Tyrode (HBT) solution and stimulated with each lysoPS analogue in the presence of concanavalin A (100 μg/mL for rat and 10 μg/mL for mouse), which is known to cross-link the FcεRI receptor, for 15 min at 37 °C. In IgE/Ag-induced degranulation, rat peritoneal mast cells (RPMCs) were passively sensitized with 10 μg/mL anti-DNP IgE for 30 min at 37 °C. The cells were washed and stimulated with each lysoPS analogue in the presence of 100 ng/mL DNP-As for 15 min at 37 °C. The histamine content in the supernatant was determined by the fluorometric assay of Shore et al.[15] Histamine release was calculated as a percentage of the total cell content. Values for histamine release are presented as the means ± SE for several replicate experiments on different samples of pooled cells.

Lipid Extraction. Lipids were extracted from 200 μL of rat sera using Extract Cartridges (Oasis HLB, Hydrophilic−Lipophilic Balance, 30 mg, Waters). Extracted lipids were dried and sonicated with 200 μL of 0.01% BSA-HBT in serial dilutions.

Evaluation of Hypothermic Effect of LysoPS Analogues. LysoPS analogues were suspended in PBS containing 0.1% bovine serum albumin and were injected iv. Rectal temperatures were measured with a rectal probe every 5 or 10 min for 60 min.

Plasma Histamine Level. Blood was drawn from lysoPS analogue-injected mice 2 min after the injection. Histamine levels were determined using an enzyme immunoassay kit (IBL) according to the manufacturer's directions.

GPR34 Stable Transformants and cAMP, MAP Kinase, and Calcium Assays. CHO-K1 cells were transfected with flag-tagged rat/mouse GPR34-pCAGGS or empty vector using TransIT-CHO reagent (Minus). The transfected cells were treated with 750 μg/mL G418 (Calbiochem) and cells that survived the treatment were subjected to immunomagnetic positive selection (MACS, Miltenyi Biotech) using antiflag antibody. For cAMP assay, cells were cultured overnight and pretreated with 0.5 mM 3-isobutyl-1-methylxanthine (Wako). After 20 min, cells were costimulated with various concentrations of lysoPS analogues in the presence of 25 μM forskolin for 30 min at 37 °C and the accumulated cAMP concentrations were measured using a cAMP-Screen System (Applied Biosystems). Intracellular Ca^{2+} concentration was determined using a CAF-110 spectrofluorometer (JASCO) by stimulating Fura-2-loaded cells with lysoPS analogues and expressed as the ratio of emission fluorescence at 500 nm upon excitation at 340 and 380 nm. MAP kinase activation was evaluated by Western blotting analyses using antibodies for phospho-p42/p44 MAP kinase and p42/p44 MAP kinase (Cell Signaling Technology). Serum-starved cells were stimulated with lysoPS analogues for 5 min at 37 °C, and after the reactions were stopped by rapid cooling on ice, the cells were lysed with lysis buffer (10 mM Tris, 250 mM sucrose, 1 mM EDTA, 0.5% NP-40, 50 mM NaF, 1 mM Na_3VO_4, 10 μg/mL aprotinin, 10 μg/mL leupeptin, 1 mM PMSF) and an aliquot of protein (6 μg) was subjected to Western blot analysis.

Expression Analysis. Mouse peritoneal mast cells were prepared from male C57BL/6 mice. Crude mast cells were purified with Percoll gradient as previously described. For preparation of bone marrow-derived mast cells, bone marrow cells from male C57BL/6 mice were cultured for 4− 6 weeks in RPMI 1640 containing recombinant mouse IL-6. The amount of GPR34 RNA were quantified using 7300 Real Time PCR System (Applied Biosystems). The sequence of the primers for GPR34 were 5′-ATGTGGCTGTTGCAGACCTTCTA-3′ and 5′-ACACCTAGTGTCCACTTGTTTTGG-3′. The copy numbers for GPR34 were normalized by those of glyceraldehydes-3-phosphate dehydrogenase (GAPDH).

Statistical Analysis. Student's t test was used for comparisons between groups. All statistical analyses were performed by using EXCEL. A P value of 0.05 or less was considered to indicate a significant difference.

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Supporting Information Available: Effect of lysoPS analogues on mast cell degranulation; LysoPT is not cytotoxic; LysoPT has its targets on mast cell membrane. Measurement of LDH

release. Withdrawal of lysoPT from mast cell membrane with buffer containing BSA. This material is available free of charge via the Internet at http://pubs.acs.org.

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