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Article

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Non-naturally-occurring Regio Isomer of Lysophosphatidylserine Exhibits Potent Agonistic Activity Towards G Protein-coupled Receptors

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+ Equal contribution

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Abstract

Lysophosphatidylserine (LysoPS), an endogenous ligand of G protein-coupled receptors, consists of *L*-serine, glycerol and fatty acid moieties connected by phosphodiester and ester linkages, respectively. An ester linkage of phosphatidylserine (PS) can be hydrolyzed at the 1-position or at the 2-position, to give 2-acyl lysophospholipid or 1-acyl lysophospholipid, respectively. 2-Acyl lysophospholipid is in non-enzymatic equilibrium with 1-acyl lysophospholipid in vivo. On the other hand, 3-acyl lysophospholipid is not found, at least in mammals, raising the question of whether the reason for this might be that the 3-acyl isomer lacks the biological activities of the other isomers. Here, to test this idea, we designed and synthesized a series of new 3-acyl lysophospholipids. Structure-activity relationship studies of more than 100 "glycol surrogates" derivatives led to the identification of potent and selective agonists for LysoPS receptors GPR34 and P2Y10. Thus, the non-natural 3-acyl compounds are indeed active, and appear to be biologically orthogonal with respect to the physiologically relevant 1- and 2-acyl lysophospholipids.

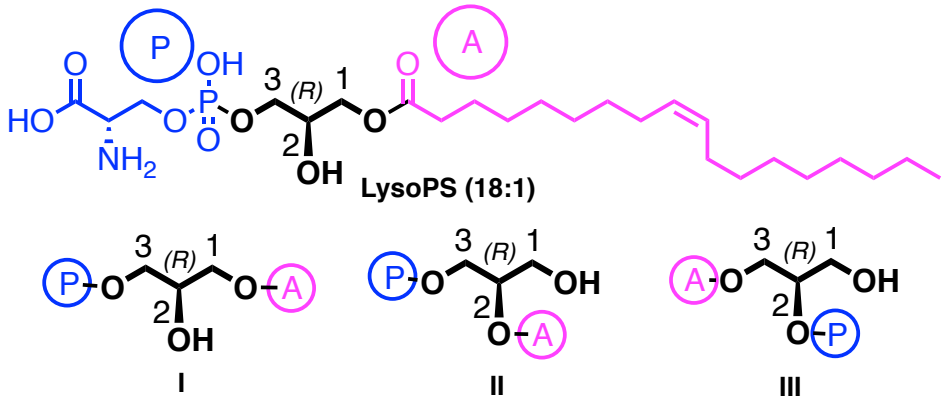
Introduction

Lysophospholipids (LPLs) and fatty acids are released by the hydrolysis of membrane phospholipids. The fatty acids serve as precursors of biologically active prostaglandins and leukotrienes, while the LPLs serve as regulators of multiple cellular responses.[1] Among the LPLs, lysophosphatidylserine (LysoPS) is thought to have a pathophysiological role in the immune system, including suppression of regulatory T cells.[2-5] Recently four G protein-coupled receptors (GPCRs), GPR34, P2Y10, A630033H20 (a non-functional truncated pseudo-gene product in human) and GPR174, have been identified as LysoPS cognate receptors and designated as LPS_1, LPS_2, LPS_{2L} (LPS_2-like) and LPS_3, respectively.[6, 7] The functional receptors are abundantly expressed in the immune systems and several organs, and studies *in vitro* and *in vivo* indicate their involvement in the control of the immune response. For example, Liebscher *et al* concluded that GPR34 is involved in immune response to antigen and pathogen challenge, based on a study of GPR34-deficient (KO) mice.[8, 9] It seems likely that dysfunction of these LysoPS receptors or dysregulation of LysoPS is linked to various diseases.

<Figure 1>

LysoPS consists of *L*-serine, glycerol and fatty acid moieties connected by phosphodiester and ester linkages, respectively. Physiologically, phosphatidylserine (PS)-specific phospholipase A_1 ($PS\text{-}PLA_1$) hydrolyzes PS regioselectively at the 1-position to give 2-acyl lysophospholipid (Figure 1(a); Type II), from which 1-acyl lysophospholipid (Type I) is formed by non-enzymatic migration of the ester.[10] Thus, Types I and II are both formed in mammals, and are biologically active.

(a)



(b)

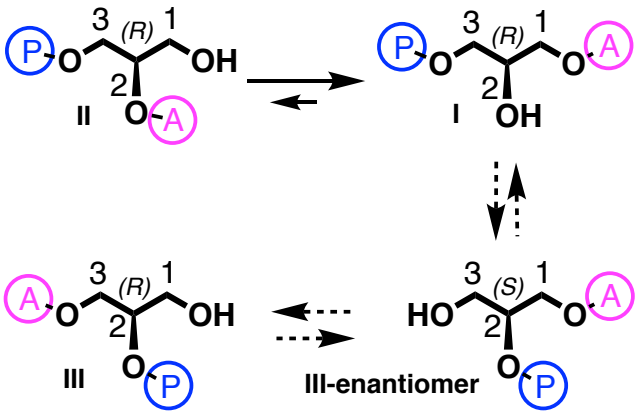


Figure 1. Geometrical Isomers of Glycerol Substitution of LysoPS (a), and Possible Mutual Transformations (b).
Types I and II are physiological, but Type III is not.

Indeed, we previously reported structure-activity-relationship (SAR) studies of synthetic LysoPS analogues of Type I and Type II and identified GPR34-, P2Y10- and GPR174-selective agonists.[11-14] On the other hand, Type III has never been reported in mammals, though it might be formed via multiple migrations of acyl and phosphodiester groups (Figure 1(b)). This raises the question of whether the reason why Type III is not produced might be that it lacks biological activity. This possibility might be consistent with the common view that when triacylglycerol (TG) is hydrolyzed in the body, only *sn*-1,2- and *sn*-2,3-diacylglycerol (DAG), not *sn*-1,3 DAG, are formed.[15] On the other hand, the formation of *sn*-1,3 DAG was argued under specified conditions.[16, 17] Therefore, Type III LysoPS derivatives are imaginable, and their biological activities are worthy to be studied.

Here, in order to answer this question, we designed and synthesized a series of new Type III LysoPS derivatives and examined their GPCR activation activities. Interestingly we found that these derivatives include potent and selective agonists for LysoPS receptors GPR34 and P2Y10, and furthermore, conformational constraint of these analogues by using a tetrahydropyran-3,4-diol framework enabled us to identify stable conformations that may be responsible for the bioactivities. We discuss the reason why these compounds are apparently not used physiologically, despite their activity.

Results and Discussion

Synthesis of LysoPS analogues

Type III LysoPS derivatives were synthesized according to established methods,[11,13,14] by the sequential buildup of protected fragments followed by global deprotection with TFA in the final step. For example, the prototype C2-derivative **1a** was synthesized as follows. Ethylene glycol was mono-acylated with oleoyl chloride. Boc-*L*-serine *tert*-butyl ester was phosphorylated with *tert*-butyl tetraisopropylphosphorodiamidite in the presence of 1*H*-tetrazole, followed by a second phosphorylation with the acylated ethylene glycol. Subsequent *in situ* oxidation of the resultant phosphite triester intermediate with *tert*-butyl hydroperoxide afforded the fully protected phosphate triester. Removal of all the protecting groups with TFA furnished the LysoPS analogue as the TFA salt. For details, see the Experimental Section.

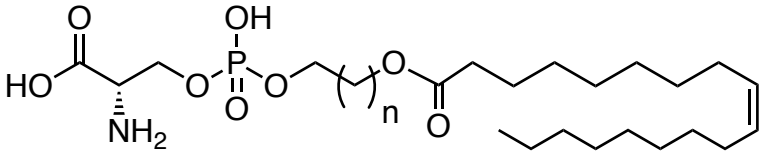
Chain-length-dependence of the activities of LysoPS analogues

We previously showed that the effects of modification of the three modules or two linkages of LysoPS, particularly Type I LysoPS (Figure 1), on the activating activity towards GPR34, P2Y10 and GPR174 are different and rather orthogonal.[11-14] For example, elimination of the hydroxyl group of the glycerol moiety, as in **1b** (1-oleoyl-2-deoxy LysoPS), diminished the GPR174 activation potency (Figure 2) without affecting the activity towards GPR34 or P2Y10. Building on that work, we focused first on synthesizing a series of Type III deoxy LysoPS analogues with different lengths of the glycerol backbone (Figure 2), that is, **1a** (deoxy-LysoPS C2 (18:1)), **1c** (deoxy-LysoPS C4 (18:1)) and **1d** (deoxy-LysoPS C5 (18:1)), wherein the fatty acid moiety is an oleoyl group (18:1). As expected, these deoxy analogues were not active towards GPR174, enabling us to focus on the activities towards GPR34 and P2Y10, which were evaluated by means of TGFα ectodomain shedding assay of alkaline phosphatase-tagged TGFα (AP-TGFα shedding assay) [7, 18] (Table 1 and Figures 3 and 4).

<Table 1> < Figures 2> <Figures 3 and 4 >

Among these 2-deoxyglycerol derivatives **1a**-**1d**, the optimal carbon chain length of the glycerol moiety was different for activities towards GPR34 and P2Y10. For GPR34, all of the C2 (**1a**), C3 (**1b**), C4 (**1c**) and C5 (**1d**) derivatives showed very weak agonistic activities compared with LysoPS (18:1) (EC_{50} = 230 nM). The EC_{50} values were as follows: **1a** EC_{50} > 3 μM, **1b** EC_{50} = 540 nM; **1c** EC_{50} = 320 nM; **1d** EC_{50} = 470 nM. In the case of P2Y10, the C2 (**1a**), C3 (**1b**) and C4 (**1c**) derivatives showed high potency (**1a** EC_{50} = 66 nM; **1b** EC_{50} = 20 nM; **1c** EC_{50} = 53 nM), similar to that of LysoPS (18:1) (EC_{50}

= 20 nM), while the C5 derivative **1d** showed weaker activity (EC_{50} = 240 nM) (Table 1 and Figures 3 and 4, and see also Supporting Information Figures S1-S2).



1a: n=1 **deoxy-LysoPS C2 (18:1)**

1b: n=2 **deoxy-LysoPS C3 (18:1)**

1c: n=3 **deoxy-LysoPS C4 (18:1)**

1d: n=4 **deoxy-LysoPS C5 (18:1)**

Figure 2 LysoPS Derivatives with Different Chain Lengths of the Glycerol Moiety

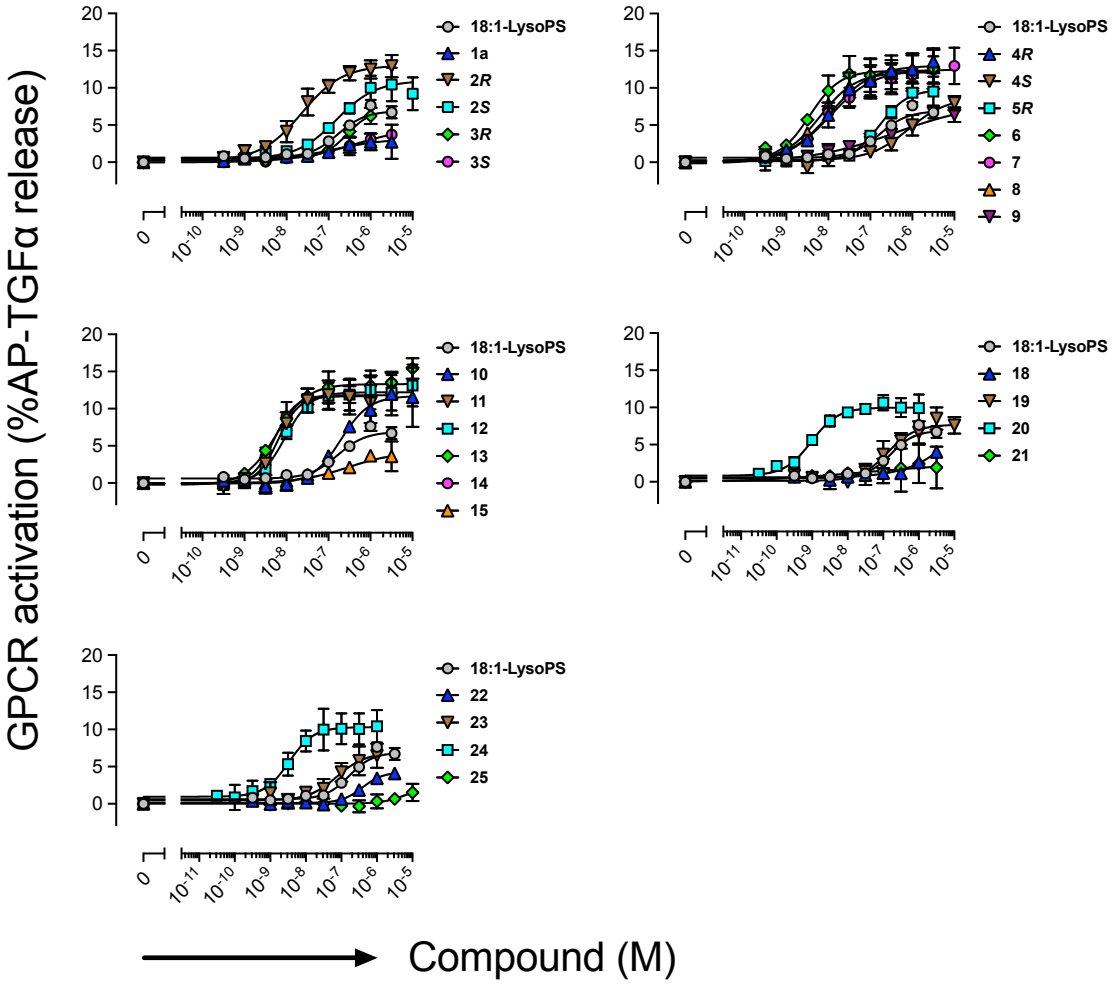


Figure 3. Concentration-response curves of GPR34 for a panel of compounds

HEK293 cells transiently transfected with expression vectors encoding alkaline phosphatase (AP)-TGFα, chimeric Gα and mouse GPR34 were treated with test compounds. Cells transfected with AP-TGFα, chimeric Gα and empty vector were used as a negative control. Receptor-specific AP-TGFα release was determined by subtracting the background responses in empty vector-transfected cells. Data are mean and SEM (standard error of the mean) for 3−8 independent experiments.

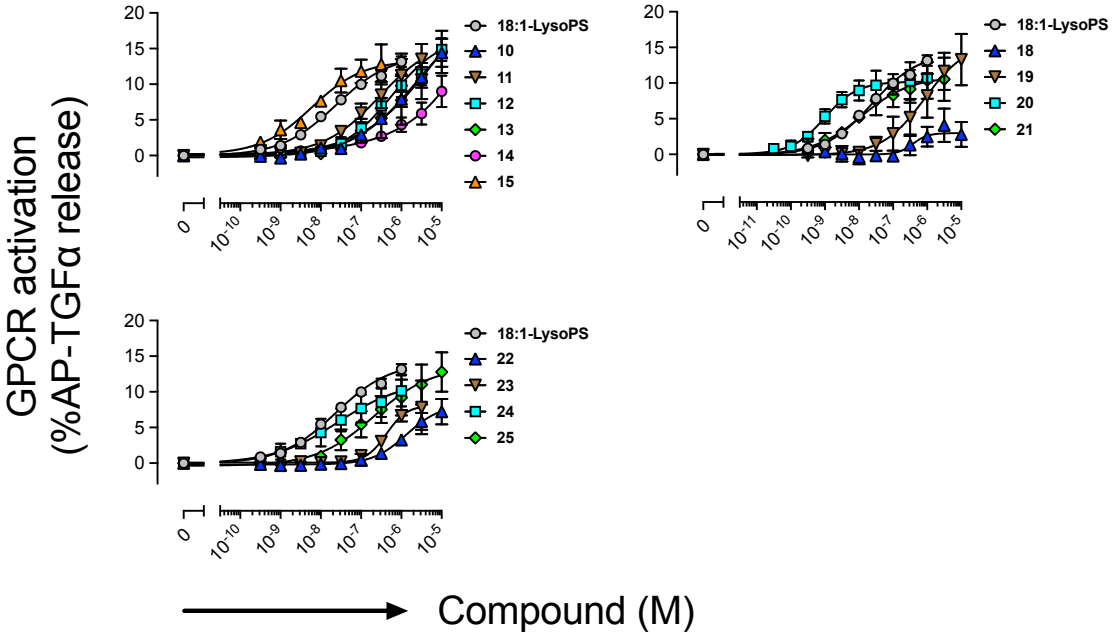


Figure 4. Concentration-response curves of P2Y10 for some compounds

Activation of P2Y10 by various compounds. Experiments were done in the same manner as in Figure 3, except that mouse P2Y10-encoding vector was used instead of mouse GPR34-encoding vector. Receptor-specific AP-TGF α release responses were determined by subtracting the background responses in empty vector-transfected cells. Data are mean and SEM (standard error of the mean) for 3−8 independent experiments.

Modification of the C2 glycerol backbone, leading to GPR34-selective agonists

Methyl Scan: The activity of deoxy-LysoPS C2 (18:1) (**1a**) toward GPR34 was exceptionally low, as compared with those of the C3 (**1b**), C4 (**1c**) and C5 (**1d**) derivatives (Table 1 and Figures 3 and 4). This suggests that just a single carbon atom deletion has a great impact on the GPR34 potency. Thus, we modified the "glycerol" backbone of deoxy-LysoPS C2 (18:1) (**1a**) by the addition of a single carbon methyl group, aiming to make it equivalent to a "C3 glycerol" skeleton (Figure 5).

<Figure 5>

There are four possible regio- and stereo-isomers (**2*R***, **2*S***, **3*R*** and **3*S***) of the methyl-substituted derivative (Figure 5), of which one, **3*R***, was examined previously.[19] The *R*-configuration is the same as the original configuration of the glycerol moiety of naturally occurring Type I LysoPS (such as LysoPS (18:1)).

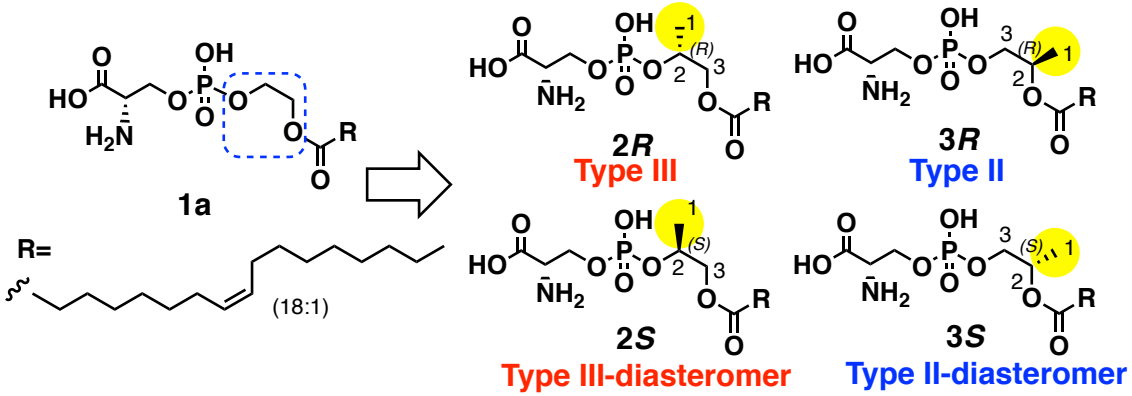


Figure 5 Methyl Scanning of the C2 Glycerol Backbone

Substitution of a methyl group at the geminal position with respect to the phosphodiester group (**2*R*** and **2*S***) increased GPR34 potency, as compared with non-methyl C2 derivative **1a** (EC_{50} > 3 μM): 1(*R*)-**2*R*** EC_{50} = 24 nM and 1(*S*)-**2*S*** EC_{50} = 170 nM (Figure 6). On the other hand, the P2Y10 potency was maintained or decreased (**2*R*** EC_{50} = 250 nM; **2*S*** EC_{50} = 37 nM) as compared with LysoPS (18:1) (EC_{50} = 20 nM). Thus, the receptor subtype selectivity of **2*R*** and **2*S*** between GPR34 and P2Y10 was moderate (Figure 6).

<Figure 6>

A methyl group at the geminal position to the fatty acid ester group (**3*R*** and **3*S***) increased the GPR34 potency of **3*R*** (EC_{50} = 250 nM) to the same level as LysoPS (18:1) (GPR34 EC_{50} = 230 nM), though **3*S*** was almost inactive (EC_{50} > 3 μM) (Figure 6). Both **3*R*** and **3*S*** showed weaker P2Y10 potency (**3*R*** EC_{50} = 79 nM; **3*S*** EC_{50} = 390 nM) than LysoPS (18:1) (P2Y10 EC_{50} = 20 nM). Thus, the stereochemistry of the methyl group markedly affects the activities towards GPR34 and P2Y10, and the compounds with the *R*-configuration, i.e., the same as the original configuration of the glycerol moiety of Type I LysoPS, are more potent than those with the *S*-configuration (Figure 6, Table 1 and Figures 3 and 4).

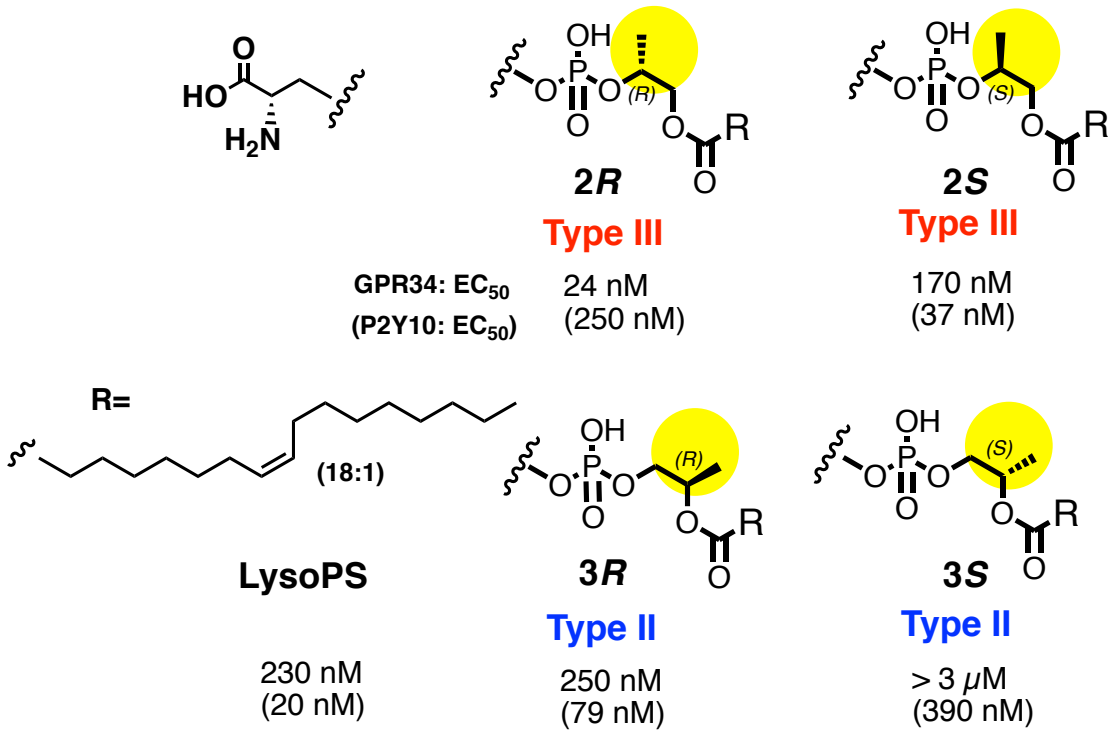


Figure 6. Receptor Activation by Methylated Type III LysoPS Derivatives

Ether substitution Next, we examined the effect of substitution with a methoxymethyl group (Figure 7). This substituent almost abrogated the P2Y10 potency of **4*R*** and **4*S*** (both $EC_{50} > 3$ μM), though **5*R*** was active ($EC_{50} = 70$ nM). As for GPR34 activation, **4*R*** exhibited slightly higher activity ($EC_{50} = 11$ nM) than the corresponding methyl-substituted analogues (**2*R***) ($EC_{50} = 24$ nM) (Figure 6), while the diastereomer **4*S*** showed reduced activity ($EC_{50} = 620$ nM) (Table 1 and Figures 3 and 4). The Type II analogue **5*R*** showed increased activity towards GPR34 ($EC_{50} = 160$ nM) as compared with the methyl counterpart (**3*R***: $EC_{50} = 250$ nM) (Table 1 and Figures 3 and 4). Based on the results for the methyl- (**2** and **3**) and methoxymethyl-substituted analogues (**4** and **5**), ***R*** configuration of the stereo center on the C2 skeleton appears to be crucial for high GPR34 potency.

<Figure 7>

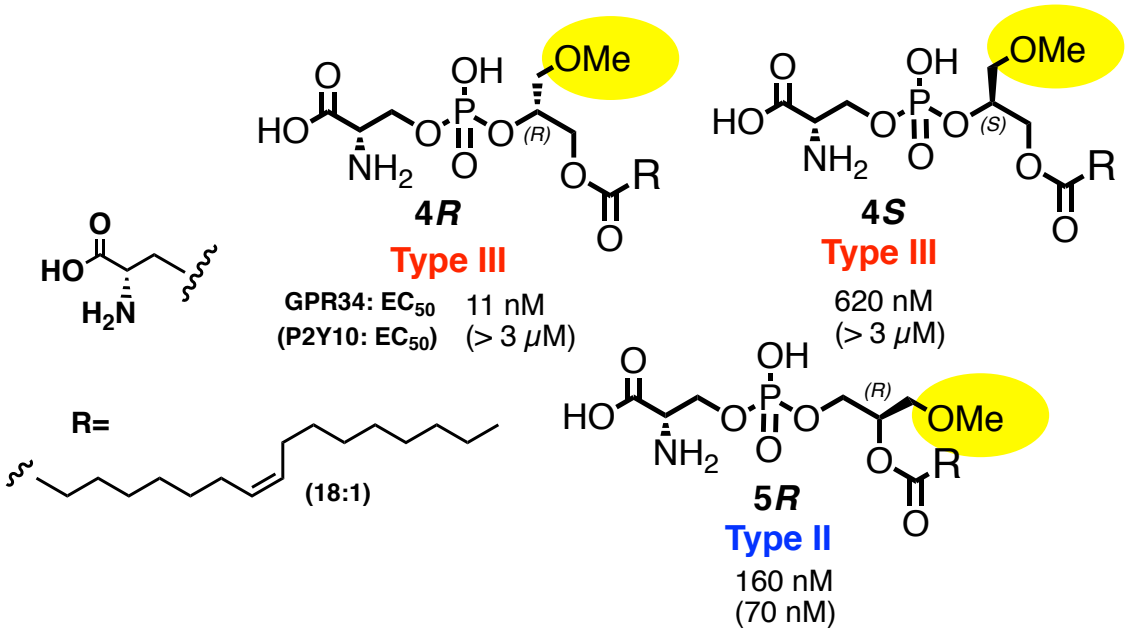


Figure 7. Receptor Activation by Ether Type III LysoPS Derivatives

Other Ether Functionalities: To examine the effect of other ether functionalities of 1(*R*)-methoxy-3-oleoyl LysoPS **4*R*** on GPR34 potency, several derivatives bearing a variety of carbon functional groups on the methoxy oxygen atom were synthesized (Figure 8). Simple alkyl ethers such as ethyl ether (**6** EC_{50} = 3.5 nM) and propyl ether (**8** EC_{50} = 6.1 nM) were found to exhibit stronger GPR34 activation ability than the simple methyl compound (**4*R*** EC_{50} = 24 nM) or unsaturated propargyl ether (**7** EC_{50} = 12 nM). In the case of the bulky benzyloxymethyl group (**9**), the GPR34 potency was significantly decreased (**9** EC_{50} = 880 nM) to a level lower than that of LysoPS (18:1). These results may indicate that there is a hydrophobic pocket in the GPR34 ligand-binding site that can accommodate glycerol backbone substituents up to a propyloxymethyl group, but not a benzyloxymethyl group, in size (see the results of the docking study below). As regards P2Y10 potency (Figure 8), simple alkyl ether derivatives such as an ethoxy and propyloxy lacked activation ability, as in the case of the methoxy compound **4*R*** (ethoxy **6** EC_{50} > 3 μM; propyloxy **8** EC_{50} > 3 μM). The unsaturated propargyl ether and benzyl ether also showed weaker activity towards P2Y10 (propargyl ether **7**: EC_{50} = 420 nM; benzyl ether **9**: EC_{50} = 1400 nM) as compared with LysoPS (18:1) (EC_{50} = 20 nM) (Table 1 and Figures 3 and 4). Therefore, the type III ethyl ether LysoPS derivative (**6**) is the most selective GPR34 agonist among the compounds studied here.

<Figure 8>

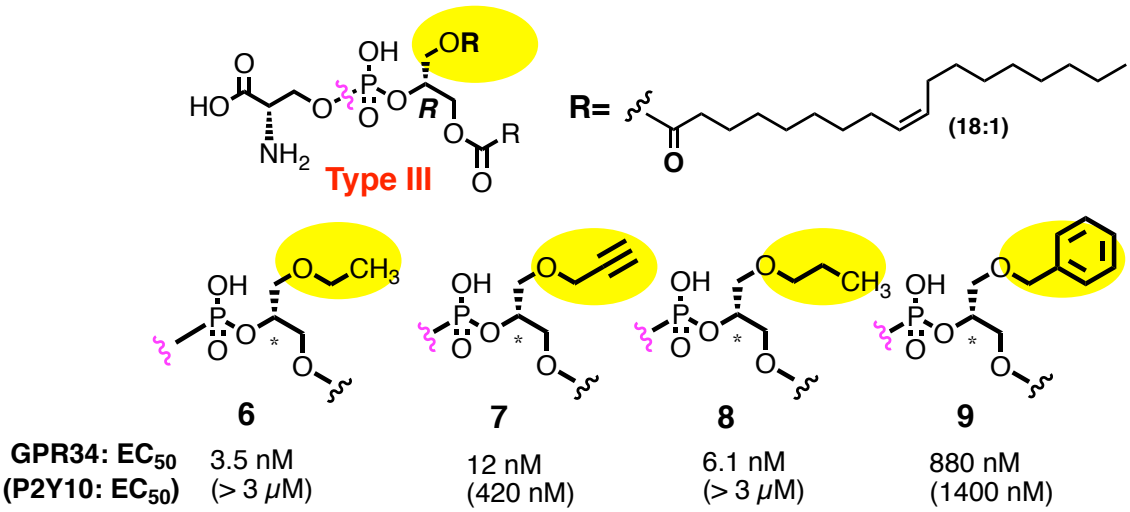


Figure 8. Ether Derivatives of Type III LysoPS

Reproducibility of methyl scanning in fatty acid surrogates

Since Type III topology of the glycerol moiety was associated with selectivity and potency for GPR34, we examined the general validity of this architecture. In our previous report,[11, 13] fatty acid surrogates including phenyl groups were found to increase the potency and selectivity of LysoPS derivatives towards specified receptors. Here, we introduced the non-fatty acid surrogates **A** and **B** (Figure 9) into the Type III lysophospholipid arrangement (Figure 10 and Table 1).

<Figure 9> <Figure 10>

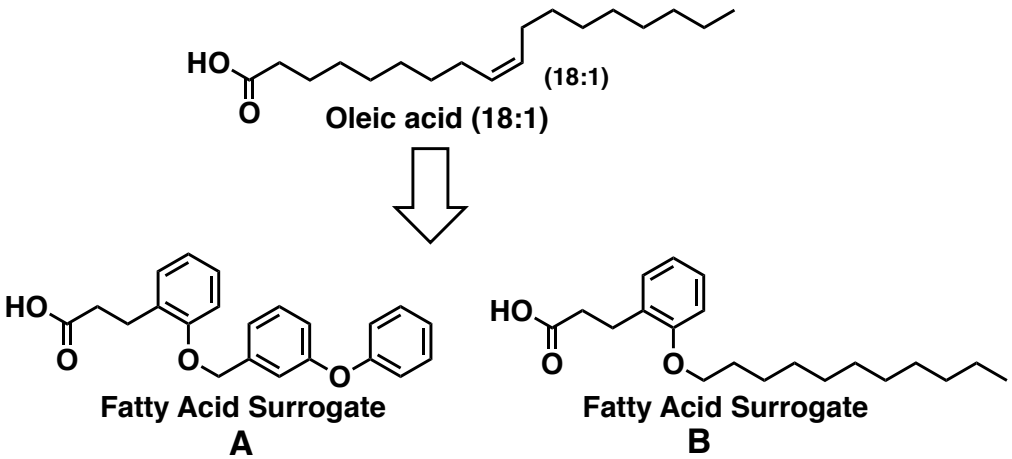


Figure 9. Fatty Acid Surrogates Used in This Work

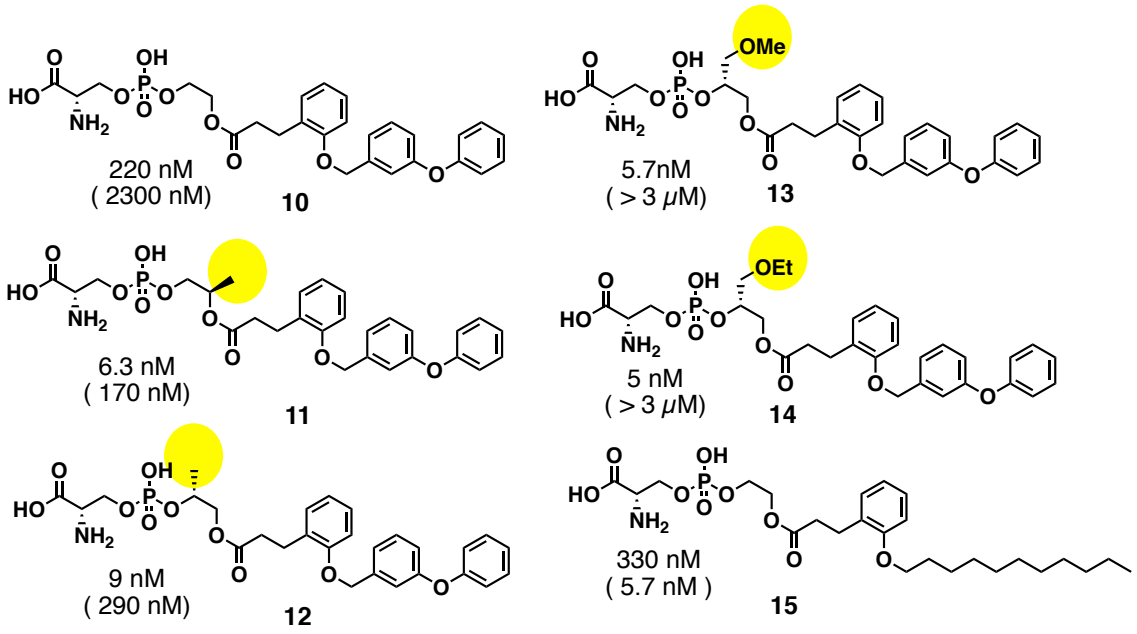


Figure 10. Structures of Type III LysoPS Derivatives Bearing Fatty Acid Surrogates

Non-lipid surrogates increased the GPR34 activity of all five derivatives **10-14** (**10**: EC_{50} = 220 nM; **11**: EC_{50} = 6.3 nM; **12**: EC_{50} = 9 nM; **13**: EC_{50} = 5.7 nM; **14**: EC_{50} = 5 nM) (Figure 10 and Table 1 and Figures 3 and 4). Among them, **14** was a selective super-agonist for GPR34 (GPR34 EC_{50} = 5 nM; P2Y10 EC_{50} > 3 μM). The C2 derivative **10** was almost inactive towards P2Y10 (EC_{50} = 2300 nM), but the methyl-substituted derivatives **11** and **12** showed activity towards P2Y10 (**11** EC_{50} = 170 nM, **12** EC_{50} = 290 nM). Both ether-type derivatives (**13** and **14**) lacked activity towards P2Y10 (EC_{50} > 3 μM), indicating that these compounds are selective agonists for GPR34.

When we used fatty acid surrogate **B** (Figure 9), the potency of **15** towards P2Y10 was greatly increased (EC_{50} = 5.7 nM), and activity towards GPR34 also emerged (EC_{50} = 330 nM), so that **15** is a P2Y10-selective agonist.

Conformational characteristics of Type III glycerol analogues

While different conformations of the glycerol unit of Types I and II LysoPS derivatives (**16**, Figure 11) can arise from the rotation of the two geminal C-C single bonds along the three-carbon chain of the glycerol moiety (C2-C3, C3-C4 in **16**, Figure 11), the conformations of the C2 glycerol surrogate of Type III LysoPS derivatives (**17**, Figure

11), can be described in terms of corresponding rotations around the two vicinal bonds along the two sets of four-atom chains, P-O-C-C (**P**) and C-C-O-C (**FA**), as shown in Figure 11.

<Figure 11>

Here, we generated accessible conformers by means of accelerated molecular dynamics calculation (replica exchange with solute tempering, REST)[20] at 300 K in the presence of explicit water (TIP3P) for 30 nsec. The obtained conformations (about 1000 snapshots obtained every 300 fsec) are plotted in terms of the two dihedral angles in Figure 10. Similar REST simulations were carried out in n-octanol, a hydrophobic environment (Figure S3).

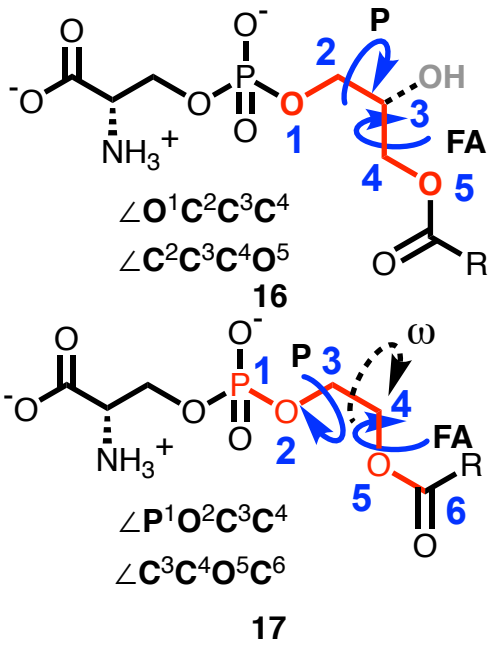
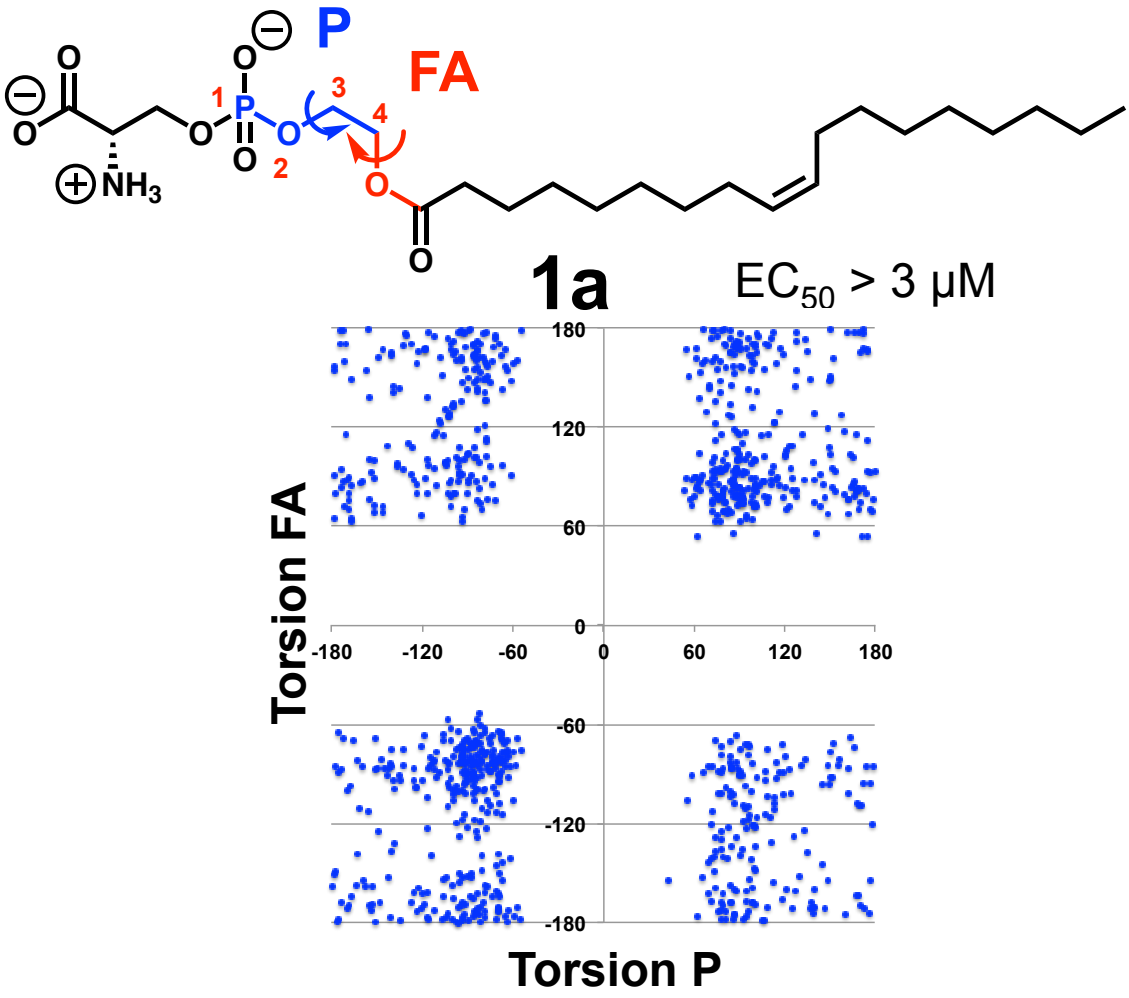


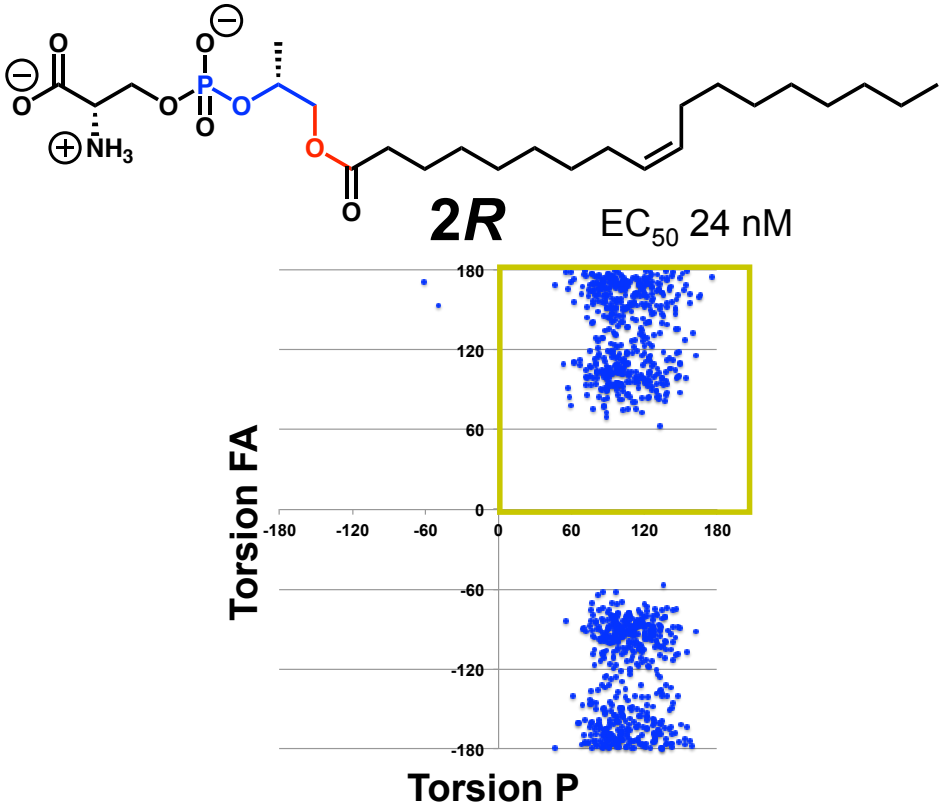
Figure 11. Two Dihedral Angles in the Glycerol Moieties

We also generated possible conformations of C2 glycerol derivatives **17** by means of accelerated molecular dynamics (MD) calculations with REST at 300 K, using the OPLS3 force field in a buffered water environment (0.15 M NaCl in TIP3P water). Sampled snapshots of the conformations were plotted with respect to the dihedral angles around the phosphate (**P**) side and fatty acid surrogate (**FA**) side. The results suggest that simple methyl substitution greatly constrains the rotation of the geminal bond, affording different distributions of conformations (Figure 12). The analysis, including cyclic derivatives (see Figure 14), suggested that the conformations in terms of these two representative dihedral angles are correlated at least to some degree with the receptor potency (*vide infra*).

Similar distributions were obtained in n-octanol (Supporting Information, Figure S3(a)). On the other hand, when we analyzed the distribution of the dihedral angle O2-C3-C4-O5 (ω) of **17** (Figure 11) and related cyclic analogues (see Figure 15), we found no correlation with the biological activities (see Figure S4).

<Figure 12>

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EC50 24 nM
Torsion FA
Torsion P
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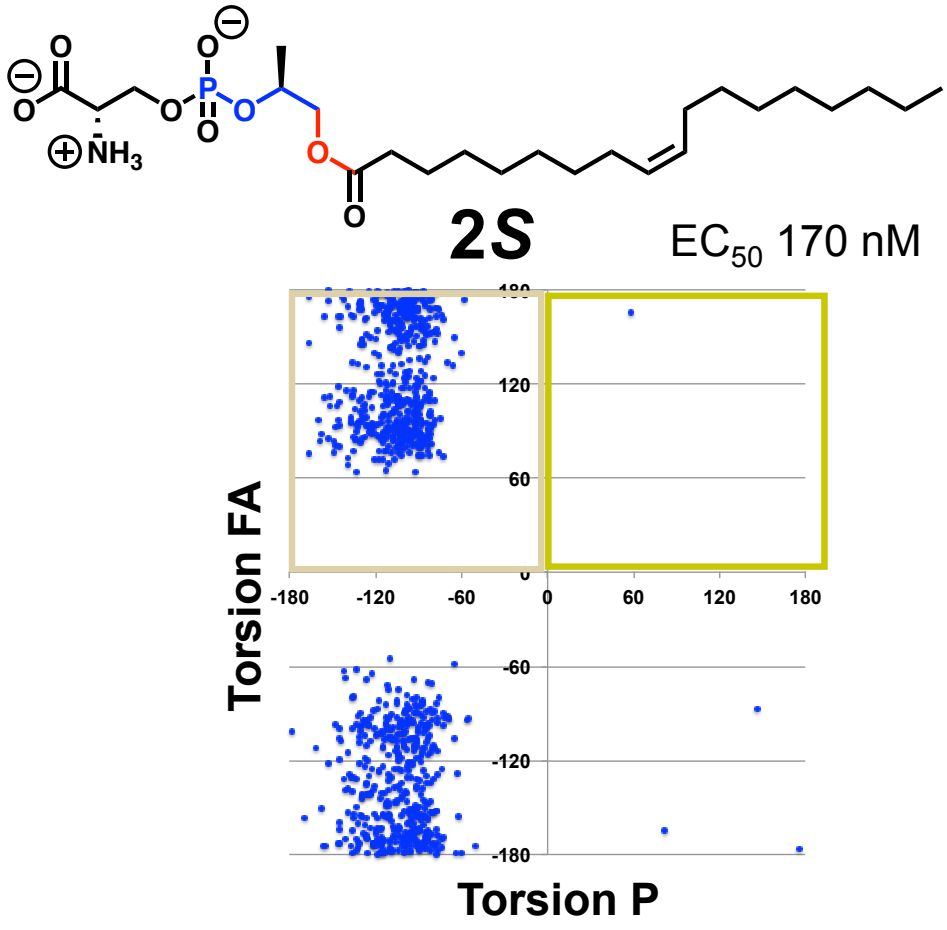
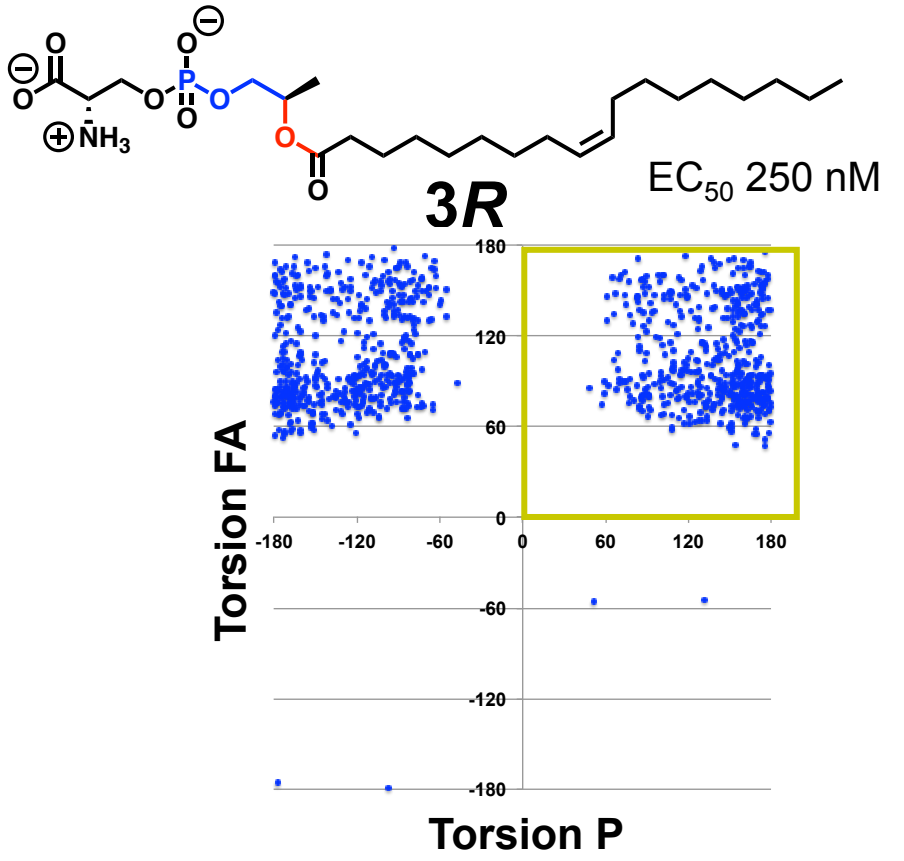
120
60
0
-60
-120
-180
-180
-120
-60
0
60
120
180


2S
EC50 170 nM
Torsion FA
Torsion P
120
60
0
-60
-120
-180
-180
-120
-60
0
60
120
180


3R
EC50 250 nM
Torsion FA
Torsion P
180
120
60
0
-60
-120
-180
-180
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60
120
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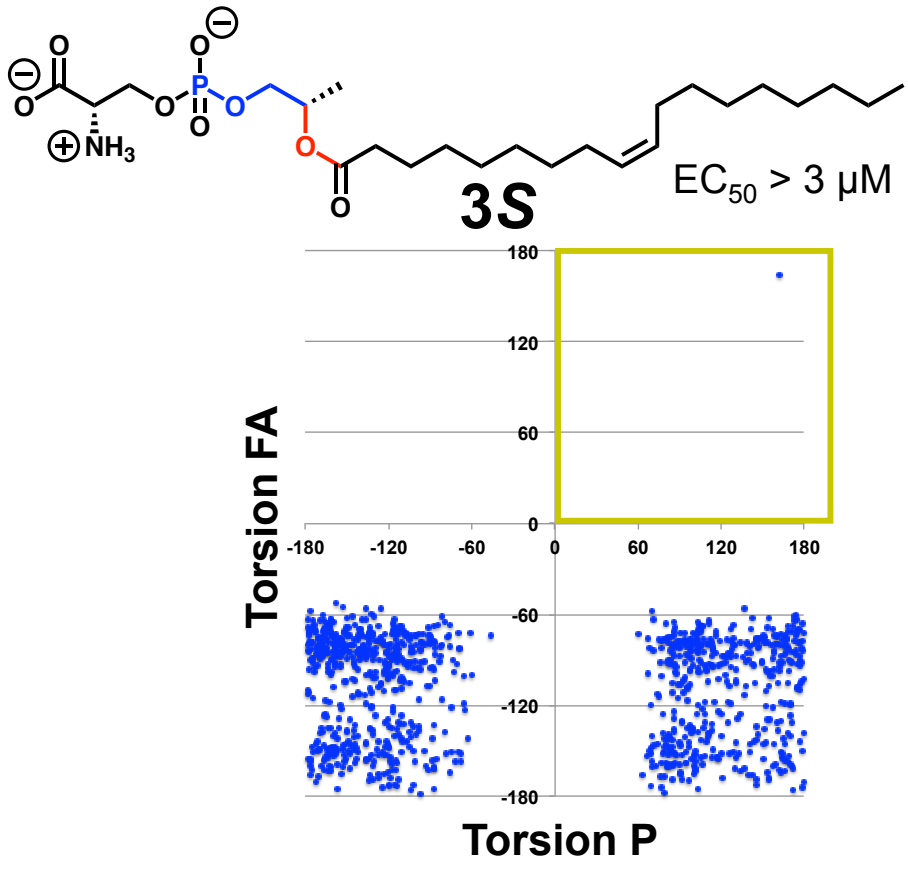

3S
EC50 > 3 μM
Torsion FA
Torsion P
180
120
60
0
-60
-120
-180
-180
-120
-60
0
60
120
180

Figure 12. Conformational distributions with respect to the dihedral angle around the phosphodiester (P) functionality and around the ester linkage of the fatty acid functionality (FA).

When the preferred conformations of the four possible isomers of methyl-substituted derivatives (**2*R*, 2*S*, 3*R*, 3*S***) were compared with each other in relation to the GPR34 potency (Figure 12), an intriguing relationship emerged: compounds exhibiting high GPR34 potency (such as **2*R***) appear to have a preference for conformations with both positive **(P)** and positive **(FA)** (Figure 12, yellow-circled quadrant). Compound **3*S***, exhibiting weaker GPR34 potency, has a higher distribution of negative **(FA)** conformations. This correlation between GPR34 activity and preferred conformation suggested that certain values of dihedral angles, i.e., **+(P)** and **+(FA)** (Figure 12, yellow-circled quadrant), are preferred for GPR34 potency (see also Figure 14). Interestingly, the dihedral angle around the fatty acid moiety **(FA)** is more critical for the GPR34 activity than that around the phosphodiester (**P**): while **2*S*** takes **-(P)** conformation on the phosphodiester side (**P**), and **3*R*** takes both +(**P**) and **-**(**P**) conformations on the phosphodiester side (**P**), the bioactivities are conserved, probably because **2*S*** and **3*R*** can both take **+(FA)** conformation on the **FA** side (see yellow-circled quadrant in Figure 12).

< Figure 12>

Conformationally constrained cyclic analogues of C2-glycerol surrogates of Type III glycerol analogues

In order to constrain the conformation of open-chain C2-glycerol surrogates such as ether **6** we utilized a cyclic tetrahydropyran-3,4-diol framework (conserving *R*-configuration at the C3 position) (Figure 13). We can define two stereoisomers of the diol as having *trans* and *cis* stereochemistry. Among eight possible isomers of LysoPS derivatives (Figure 13), we focused on four isomers of the Type III LysoPS derivatives.

<Figure 13>

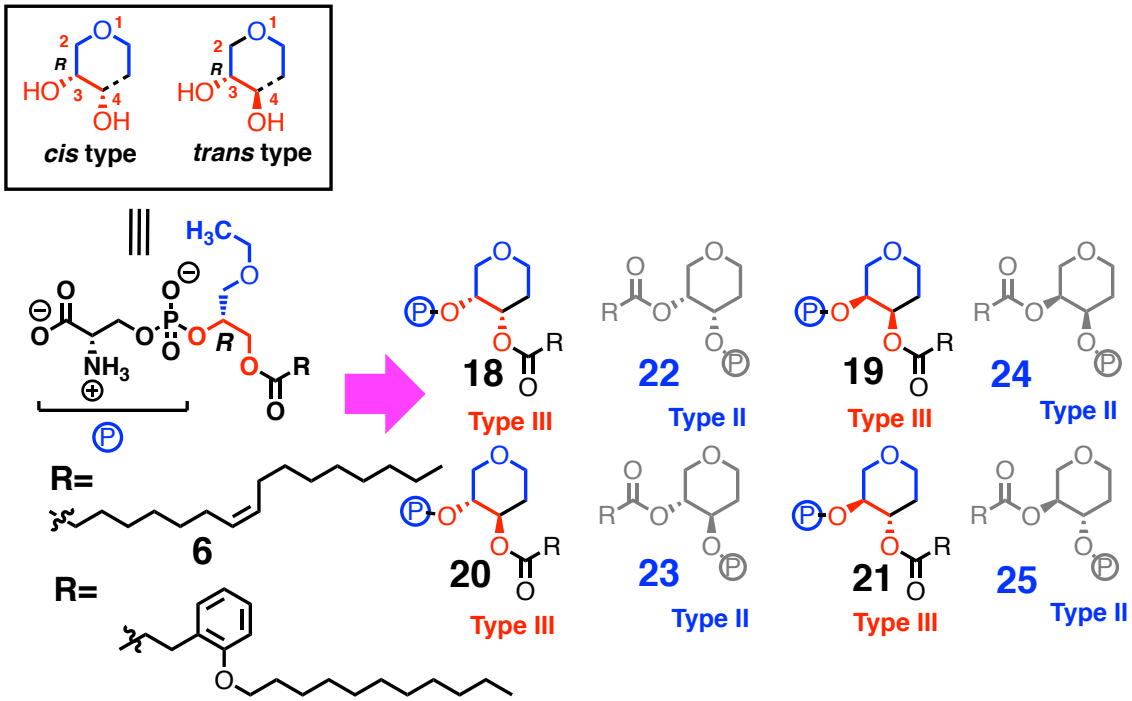
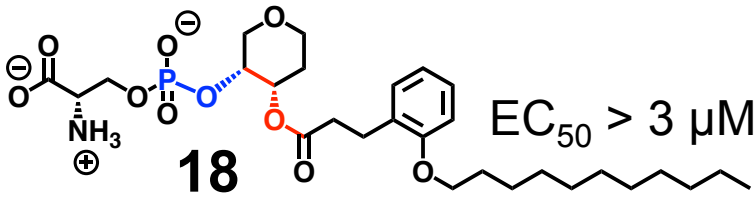
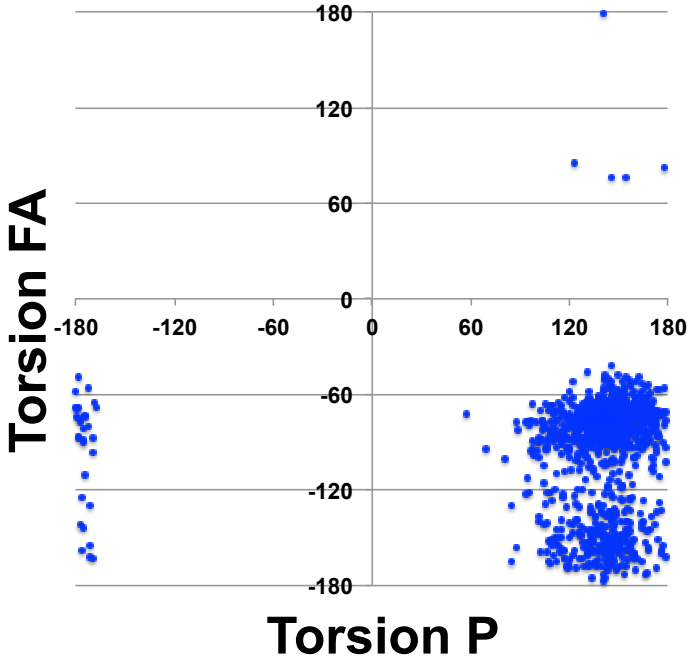


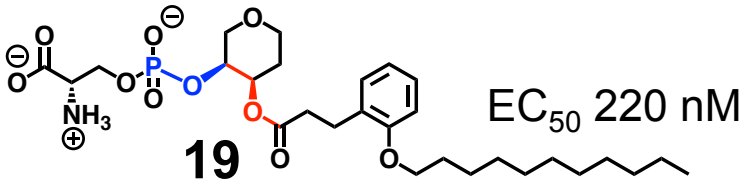
Figure 13. Design of cyclic compounds

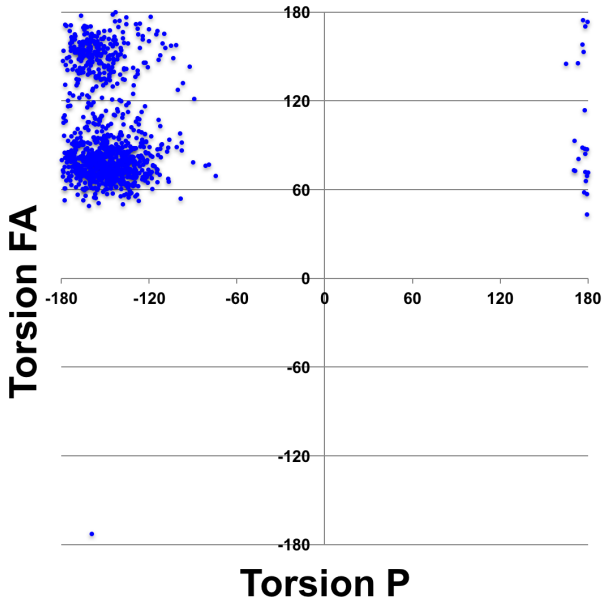
We calculated the accessible conformations of these cyclic derivatives (**18-21**) by means of REST simulations in water (TIP3P, 300 K). The distributions of conformers along the previously defined two dihedral angles around the phosphate (**P**) side and fatty acid surrogate (**FA**) side were studied in a similar manner to the open-chain analogues and the plots are shown in Figure 14. Only **20** has **+(P) +(FA)** configuration as a major structure, while **21** has **-(P) -(FA)** conformation. Thus, we predicted that **20** would show strong GPR34 agonistic activity, but **21** would be inactive towards GPR34. Also **18** has **+(P)** but **-(FA)**, and **19** has **+(FA)** but **-(P)**. Thus, we predicted that **18** would be inactive because it has **-(FA)** conformation. Similar conformational distributions were found in n-octanol (Figure S3(b)).

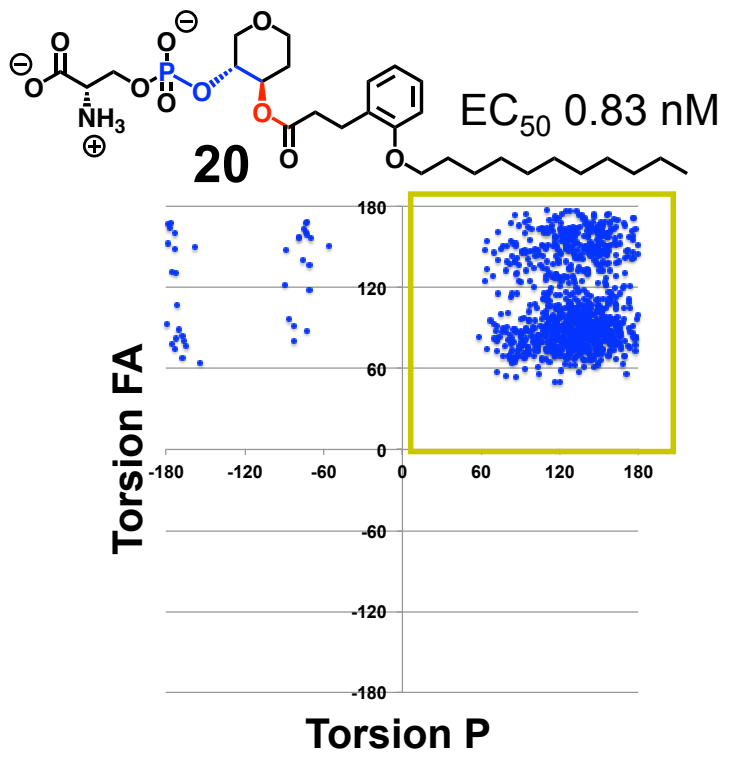
<Figure 14>


NH3
18
EC50 > 3 μM


180
120
60
0
-60
-120
-180
-180
-120
-60
0
60
120
180
Torsion FA
Torsion P


NH3
19
EC50 220 nM


180
120
60
0
-60
-120
-180
-180
-120
-60
0
60
120
180
Torsion FA
Torsion P



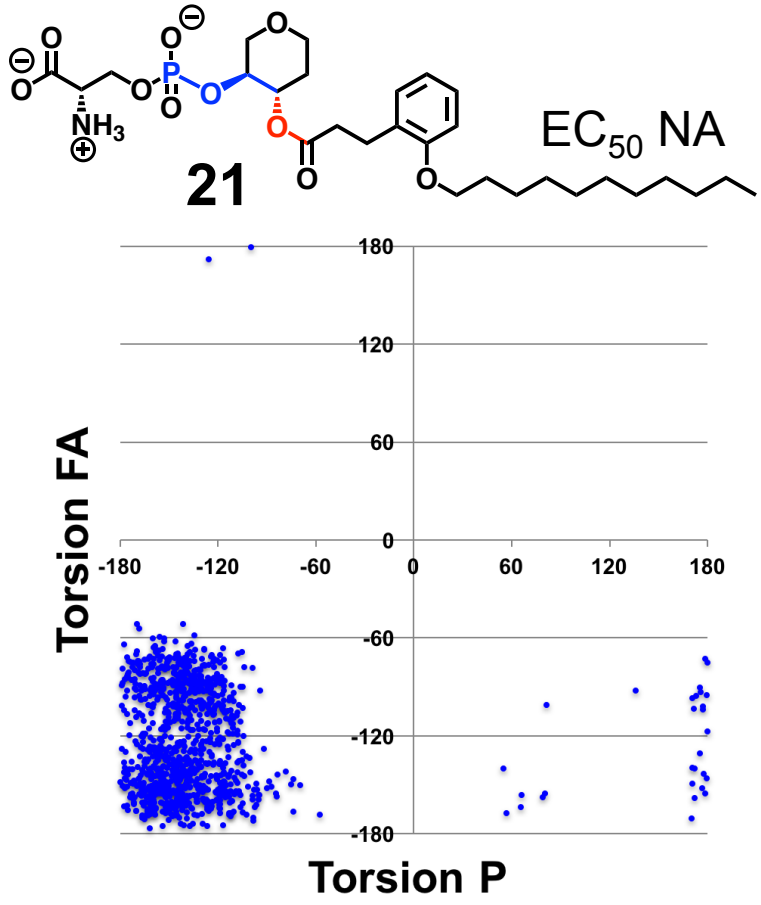


Figure 14. Simulated Conformational Preferences of Type III Cyclic LysoPS Derivatives

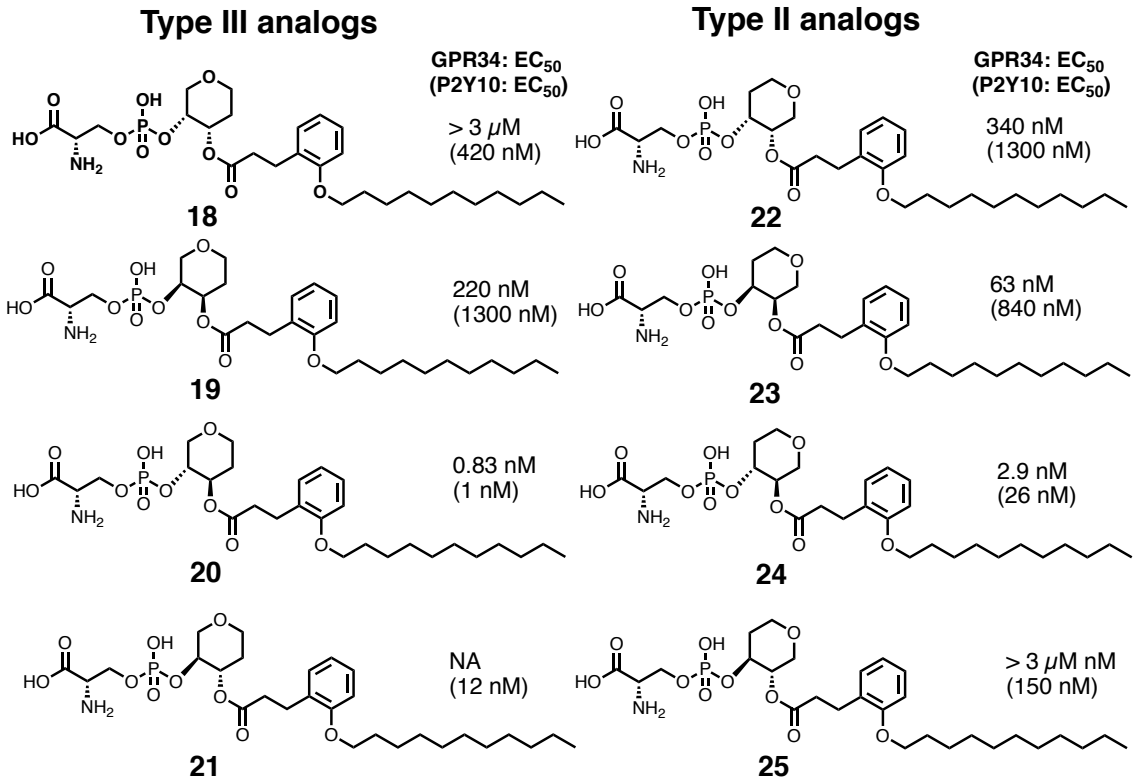


Figure 15. Cyclic Type III Analogs (18-21) and Cyclic Type II Analogs (19-25)

Eight selected conformationally constrained analogues (Figure 15), including Type III (**18**-**21**) and Type II (**22**-**25**), were synthesized as shown in Experimental Section.

<Figure 15>

The Type II (**22**-**25**) analogues and Type III (**18**-**21**) analogues have different positions of the oxygen atom in the tetrahydropyran ring, but the conformational preferences of Type II (**22**-**25**) with respect to the aforementioned dihedral angles around the fatty acid moiety (**FA**) and around the phosphodiester (**P**) should be similar to those of Type III (**18**-**21**) (Figure 14) (see Figure S11).

The tetrahydropyran-3,4-diol frameworks were synthesized from readily available monosaccharide as enantiomerically and configurationally pure carbohydrate isomers, using reported procedures.[21] The resulting diols were acylated with an aromatic non-lipid surrogate of fatty acid *C3-ph-o-O-C11* **B** (Figure 9), which was synthesized as described in our previous report.[11] The phosphotriester was constructed by the phosphoramidite

method and subsequent oxidation gave a mixture of regioisomers of LysoPS analogues in protected forms. The mixture was separated by column chromatography, and the structure of each isomer was established by means of several kinds of NMR spectroscopic analysis.

Biological evaluations of newly synthesized analogues

The results of biological evaluations of the newly synthesized cyclic analogues (**18**-**25**) are summarized in Figure 15 and Table 1 (see also Figures 3 and 4).

Type III analogues (**18**-**21**): Notably, the GPR34 potency predicted based on the conformational analysis was consistent with the biological data: while **18** and **21** are inactive towards GPR34 (**18:** $EC_{50} > 3$ μM; **21:** NA (not available: too weak to evaluate)), **20** shows potent activity ($EC_{50} = 0.83$ nM) and **19** shows moderate activity ($EC_{50} = 220$ nM). Therefore, the **+(P)** and **+(FA)** conformation of **20** appears to be crucial for activation of GPR34, as predicted. In contrast, the **-(P)** and **-(FA)** conformation as seen in **21** is inappropriate for activation of GPR34, and indeed, **21** does not activate GPR34. The dihedral angle around the fatty acid moiety (**FA**), **+(FA)** conformation, appears to be a more crucial requirement than that around the phosphodiester (**P**), **+(P)** conformation, because **19** (**-(P), +(FA)**) showed much stronger activity than **18** (**+(P), -(FA)**).

Intriguingly, while all the conformationally constrained Type III analogues **18-21** showed increased P2Y10 activity, analogues (**20** and **21**) with the *trans*-tetrahydropyran-3,4-diol framework showed higher activity than those with the *cis*-framework (**18** and **19**), and **18** and **21** are rather selective agonists for P2Y10: for P2Y10: **18** $EC_{50} = 420$ nM; **19** $EC_{50} = 1300$ nM; **20** $EC_{50} = 1$ nM; **21** $EC_{50} = 21$ nM; for GPR34: **18** $EC_{50} > 3$ μM; **19** $EC_{50} = 220$ nM; **20** $EC_{50} = 0.83$ nM; **21** $EC_{50} =$ NA.

In a previous work,[13] we found that increased planarity of the glycerol C3 conformation (using another sugar structure) resulted in increased potency toward P2Y10 rather than toward GPR34, increasing the P2Y10 selectivity. By analogy with that idea, it seems reasonable that the critical difference between compounds **14** and **20** lies in the structure of the C2 "glycerol" moiety: the increased conformational constraint in **20** may increase the planarity and the potency toward P2Y10, so that **20** becomes a dual agonist of GPR34 and P2Y10.

Type II analogues (**22**-**25**): The cyclic Type II analogues (**22**-**25**) have rather similar trends in biological activities (Figure 15 and Table 1 and Figures 3 and 4), depending on the relevant dihedral angles (Figure S5), to those found for the Type III (**18**-**21**) counterparts. The **+(P)** and **+(FA)** conformation is again crucial: **24**, corresponding to **+(P)** and **+(FA)** conformation (Figure S11), showed very potent activation of both GPR34 and P2Y10 (GPR34: EC_{50} = 2.9 nM; P2Y10: EC_{50} = 26 nM). In contrast, **25**, which has (**-(P), -(FA)**) conformation (Figure S5), showed significantly reduced potency towards GPR34 and a slightly weaker activity towards P2Y10 (GPR34: EC_{50} > 3 μM; P2Y10: EC_{50} = 150 nM). Other conformations, (**-(P), +(FA)**) and (**+(P), -(FA)**) are seen in **22** and **23**: **22** showed GPR34 EC_{50} = 340 nM and P2Y10 EC_{50} = 1300 nM, while **23** showed GPR34 EC_{50} = 63 nM and P2Y10 EC_{50} = 840 nM. Therefore, again, **+(FA)** conformation is crucial for biological activity, particularly for GPR34. Thus, the **+(P)** and **+(FA)** conformation, or at least the **+(FA)** conformation, is crucial for GPR34 agonistic activity, with the former conformation resulting in much greater potency than the latter. Among the cyclic LysoPS derivatives, both Type II and Type III derivatives can take this important **+(P)** and **+(FA)** conformation, and indeed, both show potent agonistic activities towards GPR34.

Ca^{2+} mobilization assay LysoPS was reported to induce a rapid increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in HEK293 cells transiently expressing GPR34, which is coupled with G-protein Gαi.[7] In order to confirm the potency of our LysoPS analogues toward GPR34 observed in the TGFα-shedding assay, we also used this Ca^{2+} mobilization assay. The results are summarized in Figure 16. Although there was some difference in the rank order of potency between the two assays, the results of the Ca^{2+} mobilization assay were broadly consistent with those of TGFα-shedding assay. Among the conformationally constrained analogues, **20** expressed higher Ca^{2+} mobilization activity than *cis*-type analogue, while **21** showed almost no activity. Thus, the results of both assays support our conclusions.

<Figure 16>

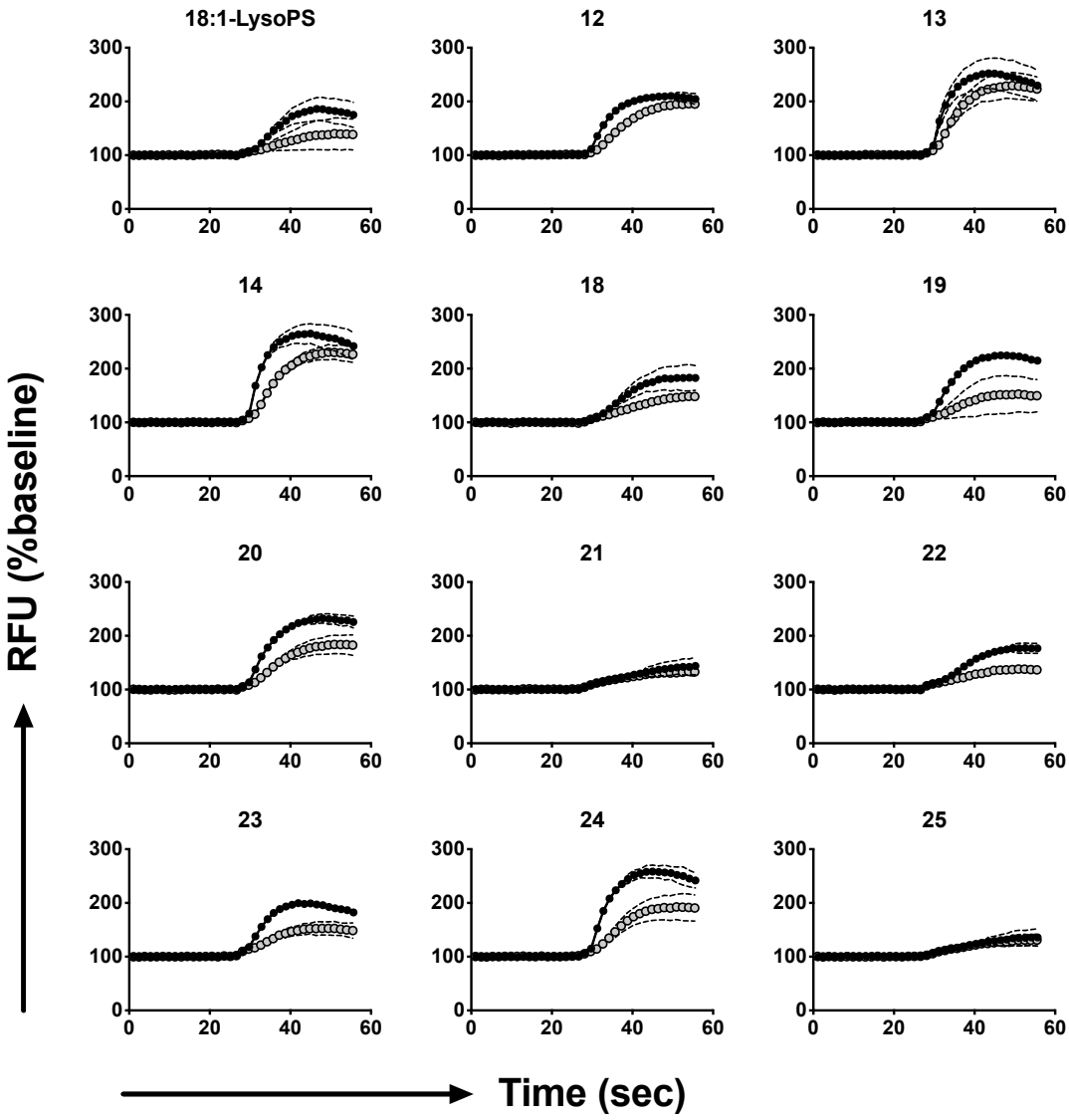


Figure 16. Calcium mobility assay

Calcium influx upon addition of each compound in mouse GPR34-expressing HEK293 cells is shown. Each experiment was performed in triplicate, and solid and dashed lines indicate the mean and the S.D., respectively. Black circles: 100 nM, gray circles: 10 nM.

Docking Study of Type III LysoPS derivative onto GPR34

A binding model of a Type I agonist to GPR34 was previously created by homology modeling, and the binding pose was evaluated and validated by means of SAR studies.[14] Since no crystallographic analysis of GPR34 has yet been reported, we used the reported GPR34 model[14] to carry out a ligand-docking study of a Type III agonist. The docking site of the Type III agonist (**14**) is tentatively assumed to be similar to that of the Type I agonist (see Figures S6 and S7).

There seems to be sufficient space between the ligand glycerol moiety and the receptor transmembrane helices (TMs) 4 and 5 for the Type III ligand to be accommodated at this site (Figure 17). This is consistent with the experimental SAR finding that Type III LysoPS derivatives, particularly those with short alkyl ether groups, show potent biological activities, which should reflect increased binding affinity. This docking model is also consistent with the existence of a small hydrophobic pocket in GPR34 that serves as a ligand-binding site with sufficient space to accommodate a propyloxymethyl group (like **6**), but not a benzyloxymethyl group (like **9**) on the glycerol backbone (see Figure 17 and Figure 8).

<Figure 17> <Figure 18>

High-affinity compounds such as **14** are expected to show stable binding poses, that is, in perturbation by means of REST calculations, the binding poses in terms of the two dihedral angles will be well conserved (Figure 18). In the case of the potent agonist **14**, these dihedral angles are distributed mainly into the (+**(P)**, +**(FA)**) and (+**(P)**, -**(FA)**) conformational regions in water, implying that molecules in the (+**(P)**, +**(FA)**) conformational region are indeed available for binding to GPR34 (Figure 12). Formation of conformations in this region was also found in the cases of strong GPR34 agonists **2*R*** and **20** (see also Figure S6).

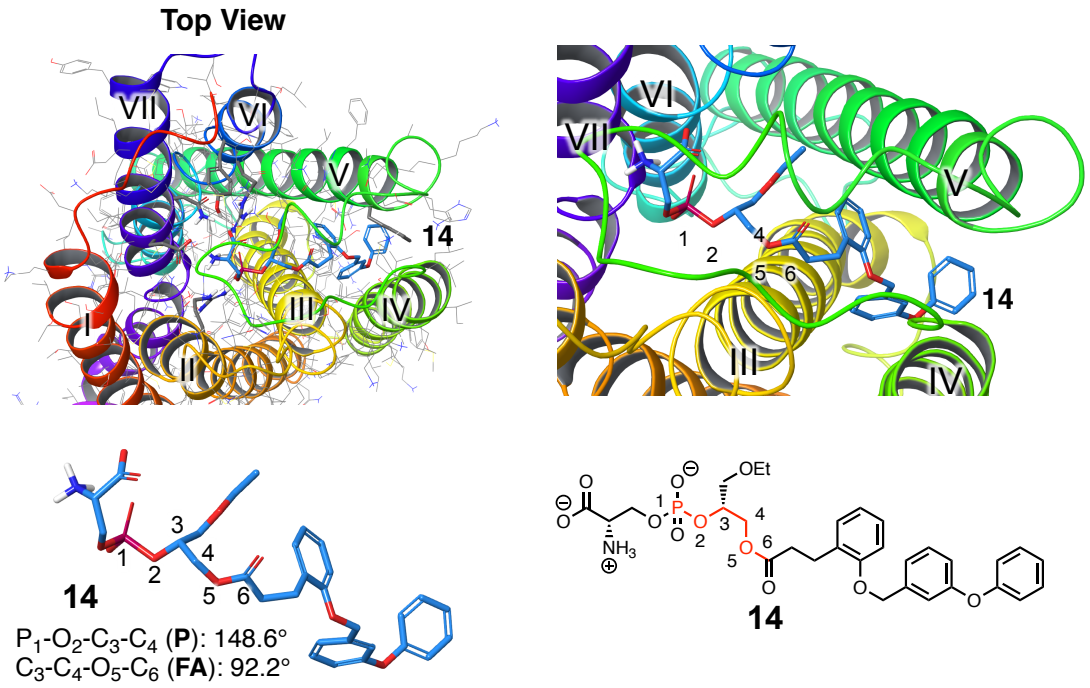


Figure 17. Binding model of Type III agonist (14) with GPR34. Space-filling by the ethoxy ether group results in tight contact with TM4 and TM5.

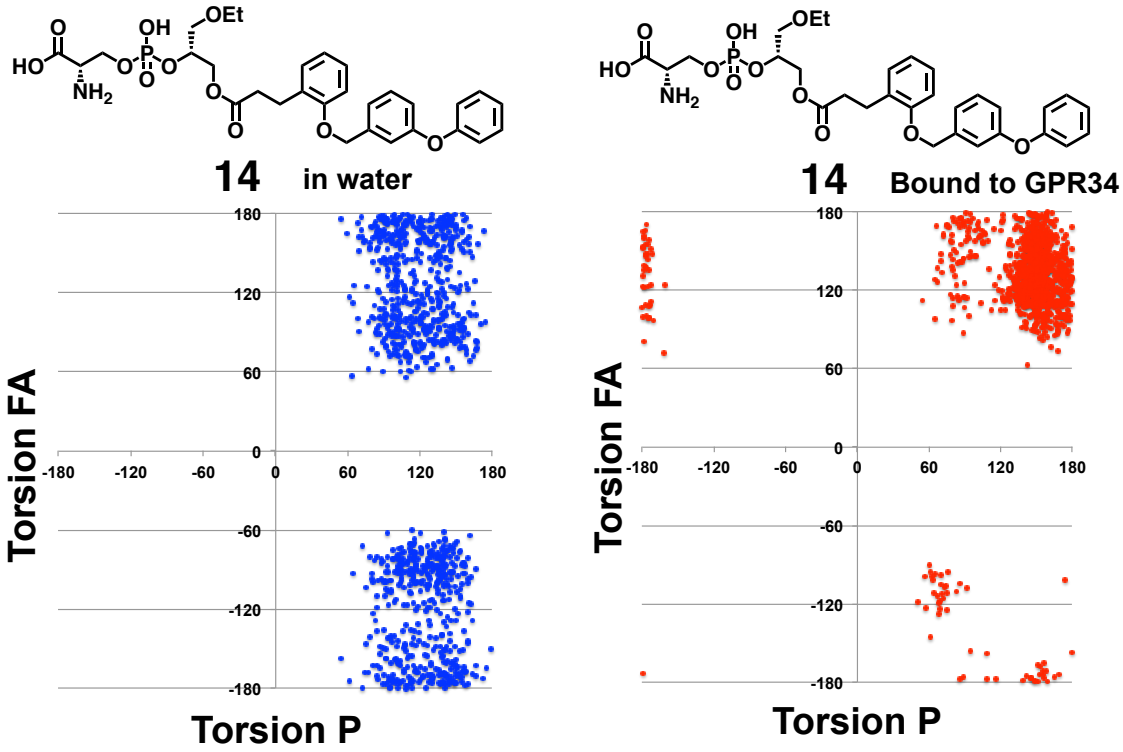


Figure 18. Converging conformational distribution upon binding of potent agonist 14 to GPR34 receptor. In the REST calculations, the distribution of conformations with respect to the two dihedral angles of 14 in water converged towards a single region (+(P), +(FA)) upon binding to GPR34

Supporting evidence for the docking study: divergent responses of GPR34 homologues to compounds 14 and 20

Genes encoding GPR34 are conserved in a wide range of vertebrates, including zebrafish.[1] While GPR34 proteins show high levels of homology with > 90% amino acid identity in mammals, only ~50% of the amino acids are conserved in zebrafish.[1] When we aligned the amino acid sequences of GPR34 from human, mouse and zebrafish (note that there are two zGPR34, types a and b), we noticed that the amino acid identities in TMs 4 and 5 are low, compared with other TMs (Table 2). Since GPR34 responds to LysoPS (18:1) across species, amino acid residues important for the recognition of LysoPS (18:1) should be conserved among species. We examined the agonistic activities of Type III compounds **14** and **20** towards zGPR34 types a and b. Although compounds **14** and **20** showed potent agonism at mammalian GPR34, they were poor agonists for zGPR34s (Table 3, Figure 19 and Figure S8). Since the amino acid sequences of transmembrane domains 4 and 5 (TMs 4 and 5) are relatively poorly conserved among

species (Figure S9: alignment), it seems plausible that the difference in activities between Type I LysoPS (18:1) and Type III compounds (**14** and **20**) could be related to the difference in TMs 4 and 5. TMs 4 and 5 are located close to the fatty acid moiety and the glycerol moiety of the ligand, respectively, in the homology model, which would be consistent with this idea. Thus, some amino acids in the TM4 and TM5 in mammalian GPR34, which are not conserved in zGPR34 type a and type b, might be involved in recognizing the fatty acid surrogates and C2-based glycerol moiety of **14** and **20**. These considerations provide some support for the validity of the present homology model.

<Table 2> <Table 3>< Figure 19>

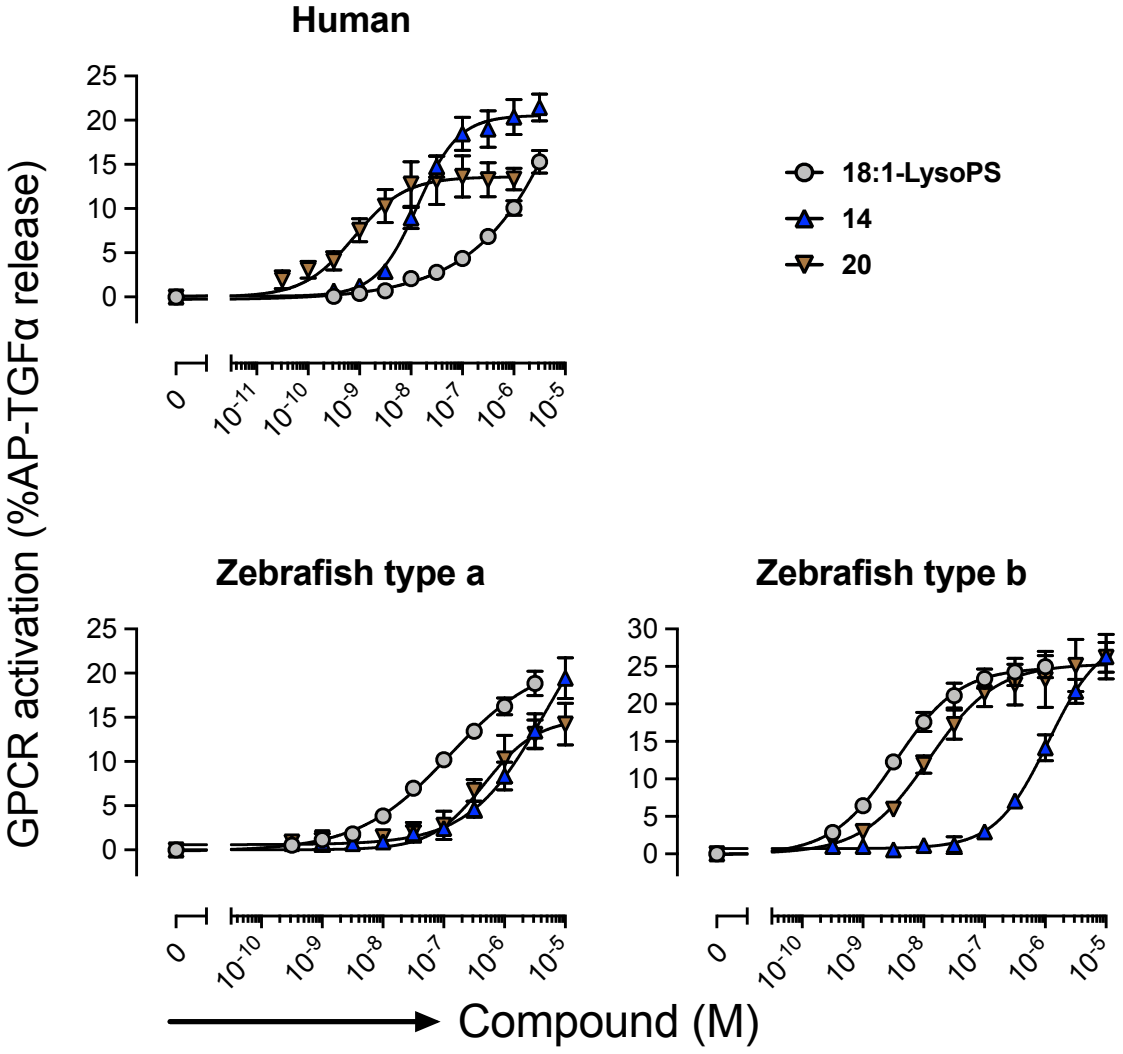


Figure 19. Differences in the potency of 18:1-LysoPS, 14 and 20 towards human and zebrafish GPR34 receptors The activation of human and zebrafish GPR34s by 18:1-LysoPS and compounds **14** and **20** was evaluated. Experiments were done in the same

manner as in Figures 3 and 4. Data are mean and SEM (standard error of the mean) for 3 −4 independent experiments.

Conclusion

In this work, we addressed the question of whether Type III LysoPS derivatives are biologically active, even though they are not found in nature. Interestingly, the biological activity exhibited a conformation dependency with respect to the C2-glycerol ("glycol surrogates") moiety that was similar to the dependency observed in physiological Type II derivatives. Conformational analysis of such compounds by molecular dynamics led to the synthesis of conformationally-constrained cyclic derivatives. The chemical work and biological results are focused on the steric requirements of receptor binding and optimization of the conformational equilibria, resulting in potent and selective ligands agonists for LysoPS receptors GPR34 and P2Y10, even although mammalians at least do not use them. This finding is intriguing in terms of molecular evolution. We suggest that only Type I and Type II LysoPS derivatives, but not Type III derivatives, may have emerged as the preferred species during evolution because Type I and II are readily inter-transformable by a single favorable chemical rearrangement, acyloxy 1,2-migration, whereas involvement of Type III would require at least two migrations, which would be relatively unfavorable, particularly the 1,3-acyloxy migration (between III-enantiomer and III, Figure 1(b)).[22] In terms of medicinal chemistry, the importance and novelty of new selective and non-selective ligands for GPCRs of lysoPS were identified, by underlining the information that was gained by conformational analysis.

Table 1. Biological assay results of Type III LysoPS Analogues[a]

	GPR34	P2Y10
	EC_{50}	
	($LogEC_{50}$)	
	[E_{max}]	
	<RIA>	
	n	
Positive Control	110 nM	1.6 nM
	(-6.96 ± 0.11)	(-8.81 ± 0.10)
	[12.2 ± 0.6%]	[11.1 ± 1.1%]
	<1 ± 0>	<1 ± 0>
	8	8
18:1-LysoPS	230 nM	20 nM
	(-6.63 ± 0.06)	(-7.71 ± 0.09)
	[8.3 ± 1.7%]	[13.6 ± 1.2%]
	<0.33 ± 0.07>	<0.083 ± 0.03>
	8	8
deoxy-LysoPS C2 (18:1) **1a**	> 3 μM	66 nM
	(> -5.5)	(-7.18 ± 0.05)
	[NA]	[10.6 ± 1.4%]
	<NA>	<0.02 ± 0.008>
	3	3
deoxy-LysoPS C3 (18:1) **1b**	540 nM	20 nM
	(-6.27 ± 0.07)	(-7.71 ± 0.10)
	[6.2 ± 1.4%]	[13.7 ± 1.4%]
	<0.12 ± 0.05>	<0.11 ± 0.01>
	3	3
deoxy-LysoPS C4 (18:1) **1c**	320 nM	53 nM
	(-6.50 ± 0.06)	(-7.28 ± 0.07)
	[4.4 ± 0.7%]	[13.1 ± 2.2%]
	<0.12 ± 0.04>	<0.029 ± 0.011>
	3	3
deoxy-LysoPS C5 (18:1) **1d**	470 nM	240 nM
	(-6.33 ± 0.07)	(-6.62 ± 0.07)
	[4.6 ± 1%]	[10.8 ± 0.8%]
	<0.076 ± 0.012>	<0.0061 ± 0.001>
	3	3
2R	24 nM	250 nM
	(-7.62 ± 0.03)	(-6.61 ± 0.08)
	[12.8 ± 0.9%]	[12.4 ± 1.5%]
	<4.8 ± 1.4>	<0.0054 ± 0.004>
	3	3
2S	170 nM	37 nM
	(-6.76 ± 0.08)	(-7.43 ± 0.06)
	[11.2 ± 1.9%]	[11.3 ± 1.2%]
	<0.56 ± 0.17>	<0.034 ± 0.02>
	3	3
3R	250 nM	79 nM
	(-6.59 ± 0.03)	(-7.10 ± 0.11)
	[6.1 ± 1.2%]	[11.4 ± 2.5%]
	<0.22 ± 0.07>	<0.02 ± 0.003>
	3	3
3S	> 3 μM	390 nM
	(> -5.5)	(-6.41 ± 0.09)
	[NA]	[11.6 ± 2%]
	<NA>	<0.0034 ± 0.0016>

	3	3
4R	11 nM	> 3 μM
	(-7.97 ± 0.10)	(> -5.5)
	[12.9 ± 0.9%]	[NA]
	<12 ± 5>	<NA>
	3	3
4S	620 nM	> 3 μM
	(-6.21 ± 0.16)	(> -5.5)
	[7.5 ± 0.2%]	[NA]
	<0.13 ± 0.06>	<NA>
	3	3
5R	160 nM	70 nM
	(-6.80 ± 0.06)	(-7.16 ± 0.62)
	[9.7 ± 1.5%]	[2.5 ± 0.8%]
	<0.52 ± 0.14>	<0.0088 ± 0.0056>
	3	4
6	3.5 nM	> 3 μM
	(-8.45 ± 0.07)	(> -5.5)
	[12.3 ± 1.4%]	[NA]
	<29 ± 6>	<NA>
	3	3
7	12 nM	420 nM
	(-7.93 ± 0.07)	(-6.38 ± 0.16)
	[12.6 ± 1%]	[3.2 ± 0.5%]
	<10 ± 4>	<0.00074 ± 0.00077>
	3	4
8	6.1 nM	> 3 μM
	(-8.21 ± 0.09)	(> -5.5)
	[12.2 ± 1.5%]	[NA]
	<17 ± 5>	<NA>
	3	3
9	880 nM	1400 nM
	(-6.05 ± 0.05)	(-5.86 ± 0.11)
	[9.6 ± 0.7%]	[7.3 ± 0.6%]
	<0.13 ± 0.02>	<0.00075 ± 0.00022>
	3	3
10	220 nM	2300 nM
	(-6.66 ± 0.13)	(-5.64 ± 0.19)
	[12.4 ± 1.9%]	[20.8 ± 1.5%]
	<0.44 ± 0.06>	<0.0013 ± 0.0004>
	3	3
11	6.3 nM	170 nM
	(-8.20 ± 0.09)	(-6.77 ± 0.13)
	[12 ± 1.1%]	[13.8 ± 0.5%]
	<16 ± 3>	<0.0099 ± 0.0057>
	3	3
12	9 nM	290 nM
	(-8.03 ± 0.13)	(-6.53 ± 0.07)
	[12.4 ± 1.4%]	[13.2 ± 2.1%]
	<11 ± 2>	<0.0056 ± 0.0018>
	3	3
13	5.7 nM	> 3 μM
	(-8.25 ± 0.06)	(> -5.5)
	[13.5 ± 0.7%]	[NA]

	<21 ± 7>	<NA>
	3	3
14	5 nM	> 3 μM
	(-8.30 ± 0.08)	(> -5.5)
	[12.1 ± 1.3%]	[NA]
	<20 ± 4>	<NA>
	3	3
15	330 nM	5.7 nM
	(-6.48 ± 0.07)	(-8.24 ± 0.11)
	[4.8 ± 0.8%]	[12.7 ± 1.3%]
	<0.12 ± 0.03>	<0.32 ± 0.05>
	3	3
18	> 3 μM	420 nM
	(> -5.5)	(-6.38 ± 0.12)
	[NA]	[3.2 ± 0.8%]
	<NA>	<0.0012 ± 0.0003>
	3	3
19	220 nM	1300 nM
	(-6.66 ± 0.17)	(-5.88 ± 0.34)
	[8.9 ± 0.9%]	[19.3 ± 3.5%]
	<0.61 ± 0.26>	<0.00089 ± 0.00442>
	3	3
20	0.83 nM	1 nM
	(-9.08 ± 0.06)	(-9.01 ± 0.14)
	[10.3 ± 0.8%]	[10.6 ± 1.4%]
	<100 ± 20>	<1.7 ± 0.1>
	3	3
21	NA [b)]	12 nM
	(NA)	(-7.91 ± 0.15)
	[NA]	[11.1 ± 2%]
	<NA>	<0.14 ± 0.01>
	4	3
22	340 nM	1300 nM
	(-6.46 ± 0.11)	(-5.88 ± 0.11)
	[3.7 ± 0.2%]	[8.6 ± 1.6%]
	<0.1 ± 0.037>	<0.00079 ± 0.00007>
	3	3
23	63 nM	840 nM
	(-7.20 ± 0.13)	(-6.08 ± 0.07)
	[6.4 ± 0.9%]	[12 ± 1.9%]
	<1.3 ± 0.5>	<0.0018 ± 0.0005>
	4	3
24	2.9 nM	26 nM
	(-8.54 ± 0.06)	(-7.58 ± 0.11)
	[10 ± 0.9%]	[11.4 ± 1.5%]
	<32 ± 12>	<0.039 ± 0.051>
	3	3
25	> 3 μM	150 nM
	(> -5.5)	(-6.83 ± 0.08)
	[NA]	[12.4 ± 1.5%]
	<NA>	<0.0085 ± 0.008>
	3	3

[a]Activities are represented in terms of EC_{50}. $LogEC_{50}$ (M) and E_{max} (% AP-TGF α release) values are calculated from the sigmoidal concentration−response curve and shown as mean ± SEM of the indicated numbers of independent experiments (n). EC_{50} values are calculated as the mean value of $LogEC_{50}$. RIA (relative intrinsic activity, a dimensionless parameter; ref 18) is a measure of agonist activity and represents the E_{max}/EC_{50} value relative to that of a positive control compound (compound **6** (*cis*-1° -(C)-2°-P) and **10b** (diF-C3-ph-*o*-O-C11) in the previous works (ref 11, 13) for GPR34 and P2Y10, respectively). By definition, the RIA value of the positive control compound is equal to 1. "NA" means "not available", because of very low activity.

Table 2. Homology among the transmembrane domains of GPR34 homologues

TM #	Human			Mouse	
	Mouse	Zebrafish type a	Zebrafish type b	Zebrafish type a	Zebrafish type b
1	94.3% (33/35)	48.6% (17/35)	40% (14/35)	51.4% (18/35)	45.7% (16/35)
2	96.7% (29/30)	53.3% (16/30)	56.7% (17/30)	53.3% (16/30)	56.7% (17/30)
3	100% (34/34)	70.6% (24/34)	70.6% (24/34)	70.6% (24/34)	70.6% (24/34)
4	88.5% (23/26)	14.8% (4/27)	23.1% (6/26)	14.8% (4/27)	30.8% (8/26)
5	97.2% (35/36)	44.4% (16/36)	36.1% (13/36)	47.2% (17/36)	38.9% (14/36)
6	100% (38/38)	60.5% (23/38)	55.3% (21/38)	60.5% (23/38)	55.3% (21/38)
7	94.4% (34/36)	55.6% (20/36)	58.3% (21/36)	52.8% (19/36)	55.6% (20/36)

Amino acid identities among each transmembrane domain (TM1-7) of GPR34 homologues are shown. In parentheses, numbers of conserved amino acids and total amino acids in each transmembrane are shown. Amino acid sequences of each homologue were aligned by using the ClustalW program and viewed with MacVector software. Predicted transmembrane domains of human GPR34 was obtained from GPCRdb (https://gpcrdb.org/).

Table 3. Potency of 14 and 20 for human and zebrafish GPR34[b]

	Human	Zebrafish type a	Zebrafish type b
		EC50 (*)	
		(LogEC50)	
		[Emax]	
		n =	
18:1-LysoPS	> 3 μM	130 nM	3.5 nM
	(> -5.5)	(-6.89 ± 0.09)	(-8.46 ± 0.01)
	[NA]	[22.1 ± 2.3%]	[25.1 ± 1.5%]
	4	4	3
14	14 nM	> 3 μM	1200 nM
	(-7.87 ± 0.03)	(> -5.5)	(-5.93 ± 0.02)
	[20.5 ± 1.3%]	[NA]	[29 ± 1.5%]
	3	4	3
20	0.75 nM	510 nM	12 nM
	(-9.12 ± 0.04)	(-6.29 ± 0.09)	(-7.94 ± 0.01)
	[13.9 ± 1%]	[14 ± 1.6%]	[25 ± 1.7%]
	4	3	3

[b]Activities are represented in terms of EC_{50}. $LogEC_{50}$ (M) and E_{max} (% AP-TGF α release) values are calculated from a sigmoidal concentration− response curve and shown as mean ± SEM of indicated numbers of independent experiments (n). EC_{50} values are calculated from a mean value of $LogEC_{50}$. "NA" means "not available" because of very low activity.

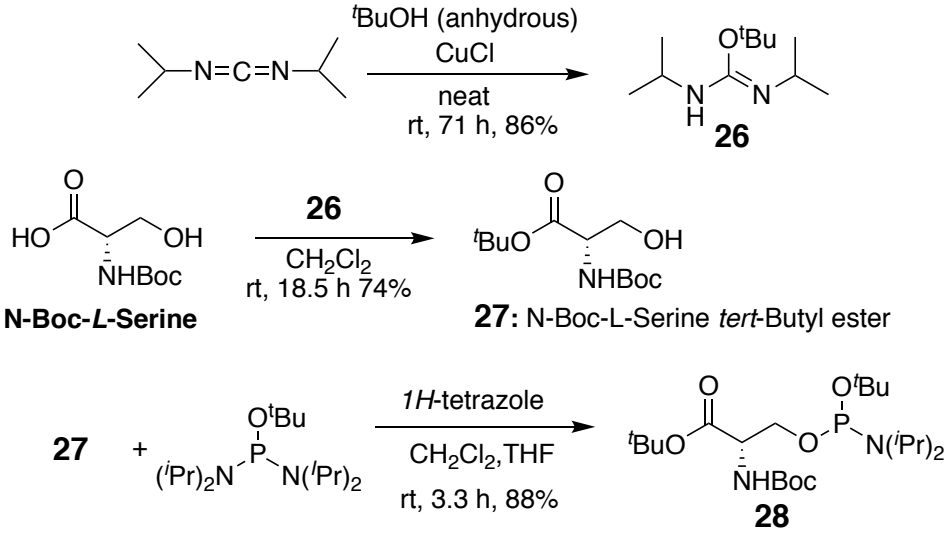


Figure 20. Synthesis of the Phosphoamidate Intermediate for the Serine Part: Synthesis of 28 (*tert*-butyl *O*-(*tert*-butoxy(diisopropylamino)phosphaneyl)-*N*-(*tert*-butoxycarbonyl)-*L*-serinate)

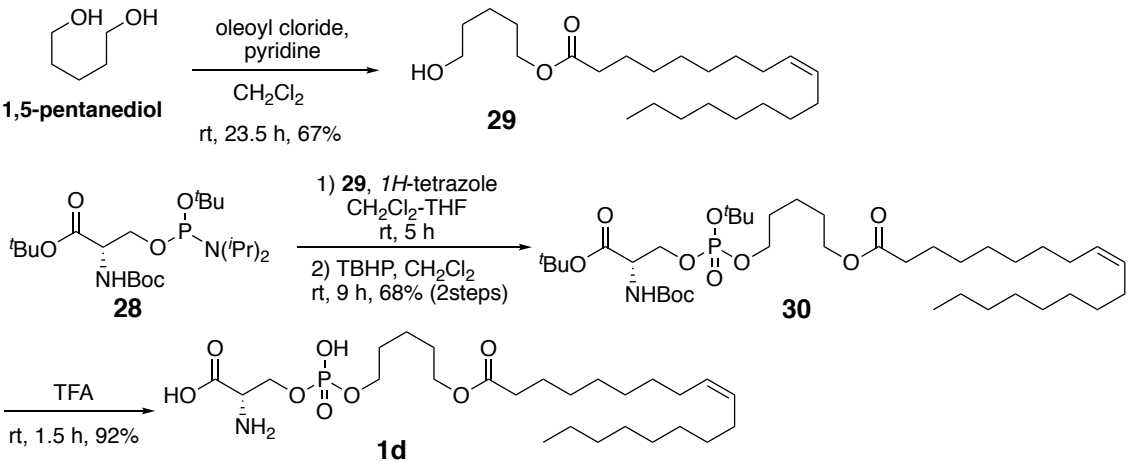


Figure 21. Synthesis of 1d (*O*-(hydroxy((5-(oleoyloxy)pentyl)oxy)phosphoryl)-*L*-serine: deoxy-LysoPS C5 (18:1))

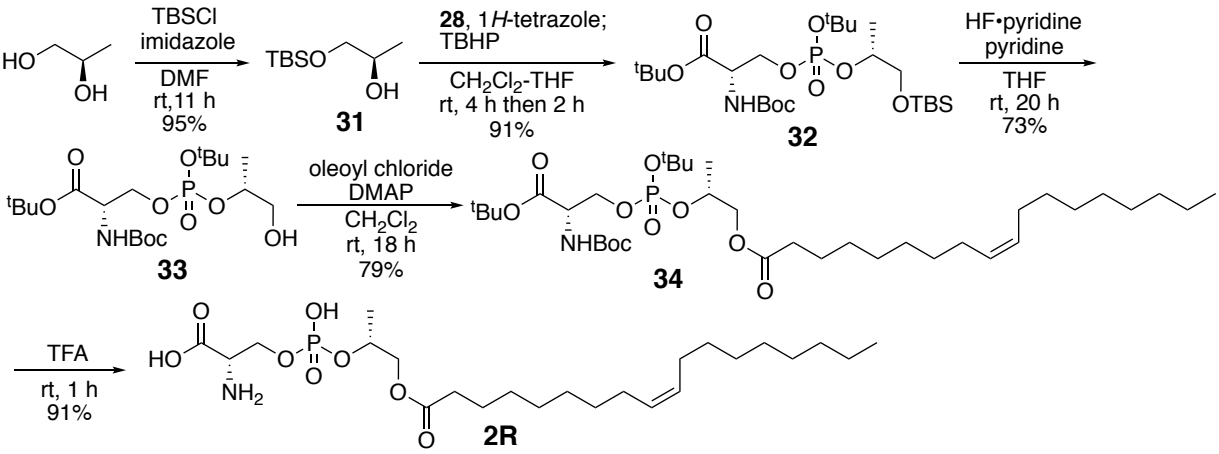


Figure 22. Synthesis of 2*R* (*O*-(hydroxy(((*R*)-1-(oleoyloxy)propan-2-yl)oxy)phosphoryl)-*L*-serine: 1-deoxy-3-oleoyl-LysoPS (R))

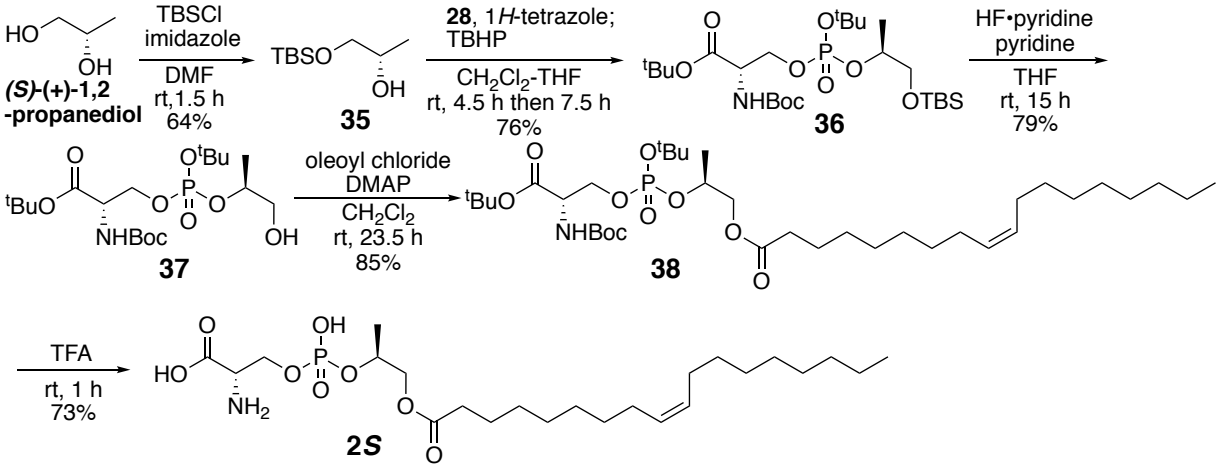


Figure 23. Synthesis of 2S (*O*-(hydroxy(((*S*)-1-(oleoyloxy)propan-2-yl)oxy)phosphoryl)-*L*-serine (1-deoxy-3-oleoyl-LysoPS (S))

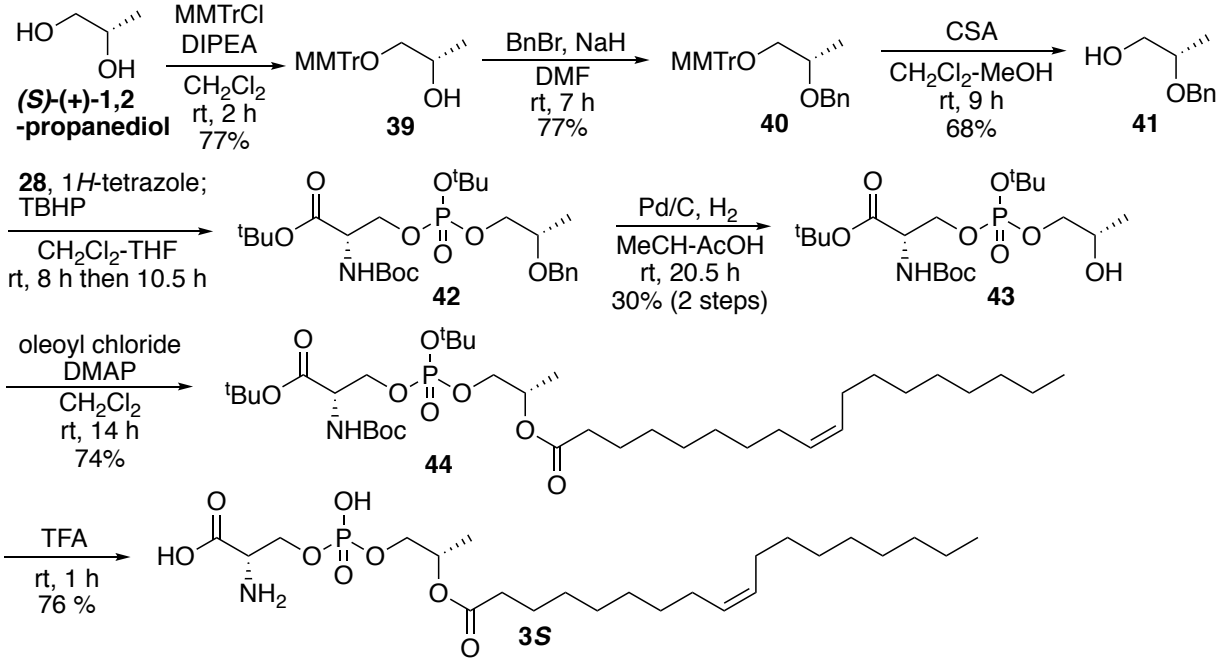


Figure 24. Synthesis of 3*S* (*O*-(hydroxy((*S*)-2-(oleoyloxy)propoxy)phosphoryl)-*L*-serine (1-deoxy-2-oleoyl LysoPS)

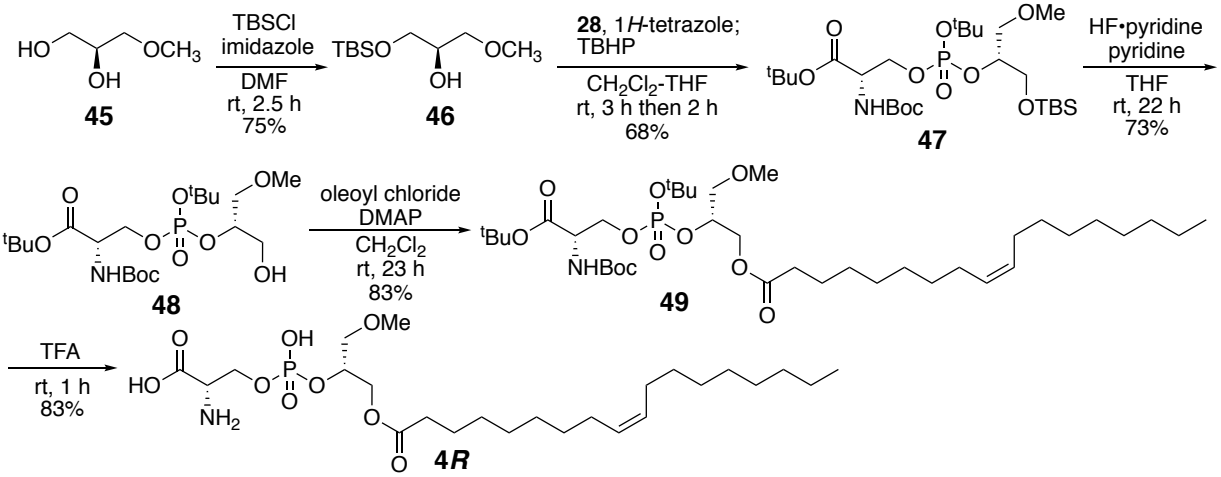


Figure 25. Synthesis of 4*R* (*O*-(hydroxy(((*R*)-1-methoxy-3-(oleoyloxy)propan-2-yl)oxy)-phosphoryl)-*L*-serine (1-methoxy-3-oleoyl LysoPS)

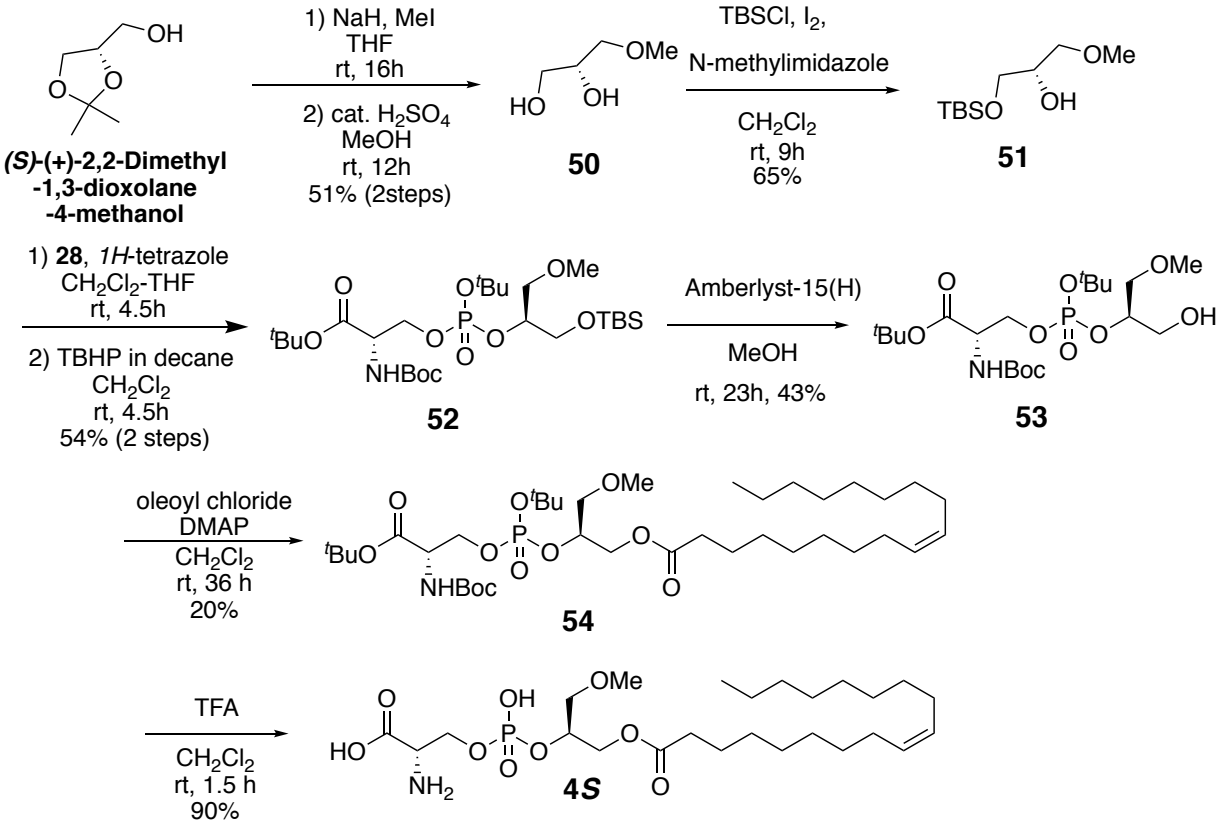


Figure 26. Synthesis of 4*S* (*O*-(hydroxy(((*S*)-1-methoxy-3-(oleoyloxy)propan-2-yl)oxy)-phosphoryl)-*L*-serine (1-methoxy-3-oleoyl LysoPS (S))

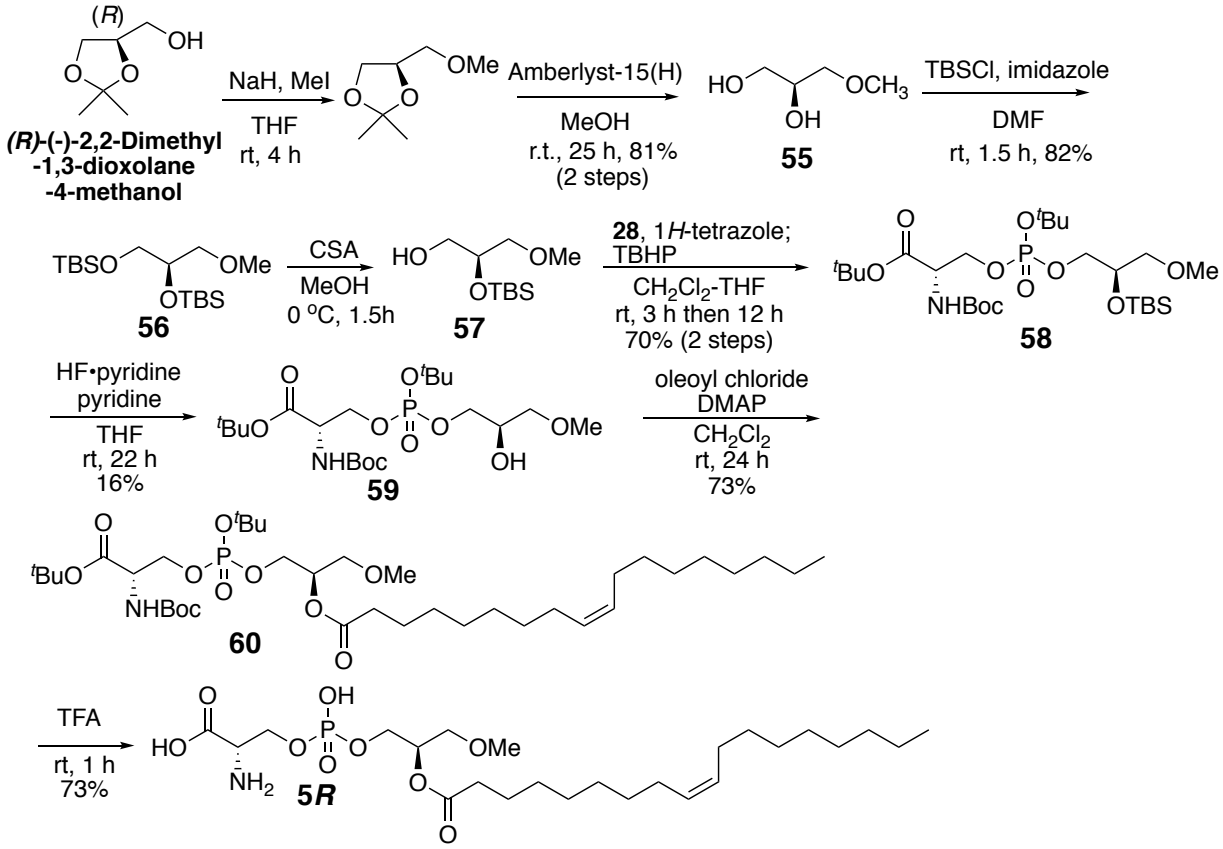
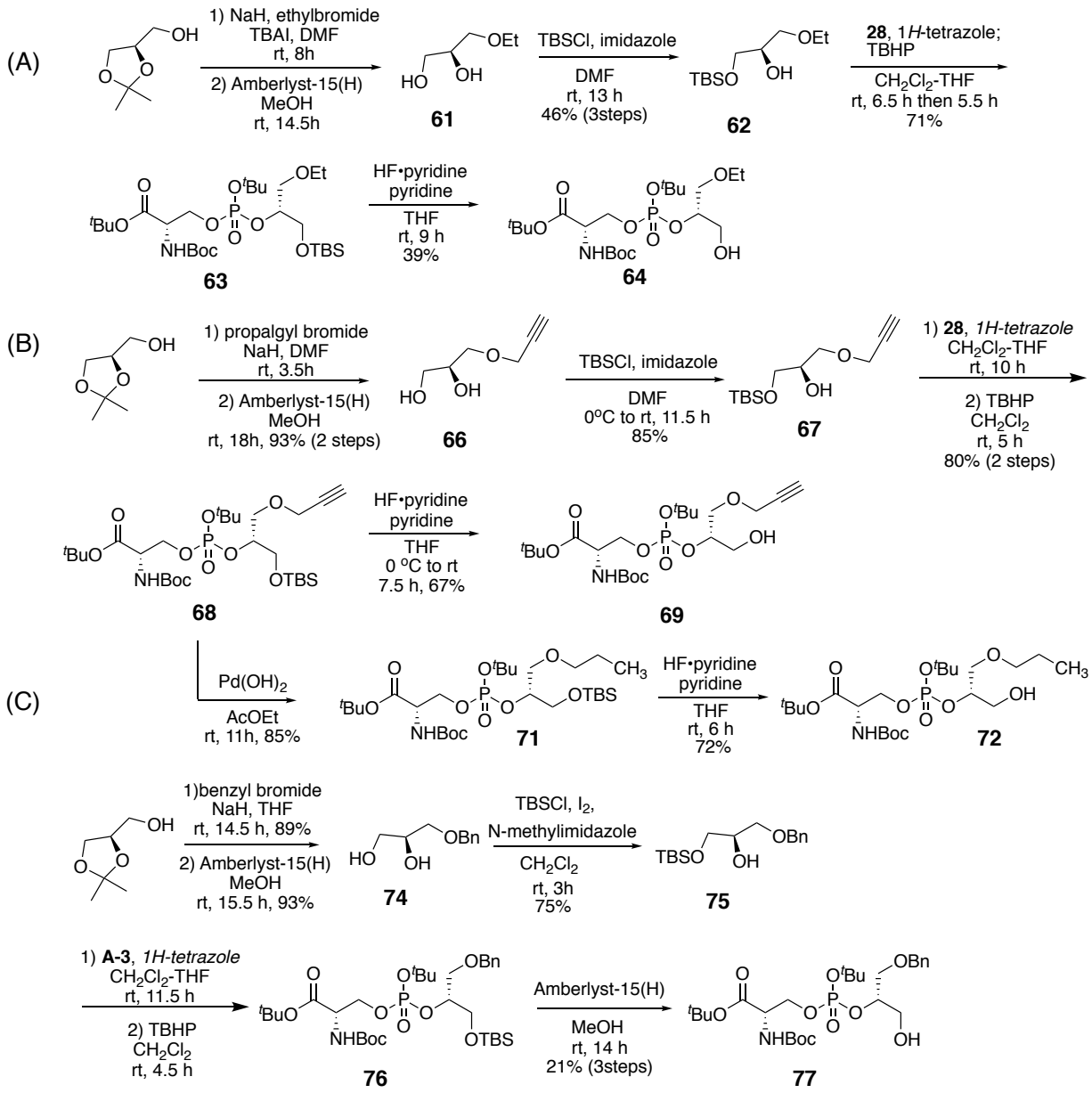


Figure 27. Synthesis of 5*R* (*O*-(hydroxy((*R*)-3-methoxy-2-(oleoyloxy)propoxy)phosphoryl)-*L*-serine (1-methoxy-2-oleoyl-LysoPS))


(A)
1) NaH, ethylbromide
TBAI, DMF
rt, 8h
2) Amberlyst-15(H)
MeOH
rt, 14.5h
61
TBSCl, imidazole
DMF
rt, 13 h
46% (3steps)
62
28, 1H-tetrazole;
TBHP
CH2Cl2-THF
rt, 6.5 h then 5.5 h
71%
63
HF•pyridine
pyridine
THF
rt, 9 h
39%
64
(B)
1) propalgyl bromide
NaH, DMF
rt, 3.5h
2) Amberlyst-15(H)
MeOH
rt, 18h, 93% (2 steps)
66
TBSCl, imidazole
DMF
0°C to rt, 11.5 h
85%
67
1) 28, 1H-tetrazole
CH2Cl2-THF
rt, 10 h
2) TBHP
CH2Cl2
rt, 5 h
80% (2 steps)
68
HF•pyridine
pyridine
THF
0 °C to rt
7.5 h, 67%
69
(C)
Pd(OH)2
AcOEt
rt, 11h, 85%
71
HF•pyridine
pyridine
THF
rt, 6 h
72%
72
1)benzyl bromide
NaH, THF
rt, 14.5 h, 89%
2) Amberlyst-15(H)
MeOH
rt, 15.5 h, 93%
74
TBSCl, I2,
N-methylimidazole
CH2Cl2
rt, 3h
75%
75
1) A-3, 1H-tetrazole
CH2Cl2-THF
rt, 11.5 h
2) TBHP
CH2Cl2
rt, 4.5 h
76
Amberlyst-15(H)
MeOH
rt, 14 h
21% (3steps)
77

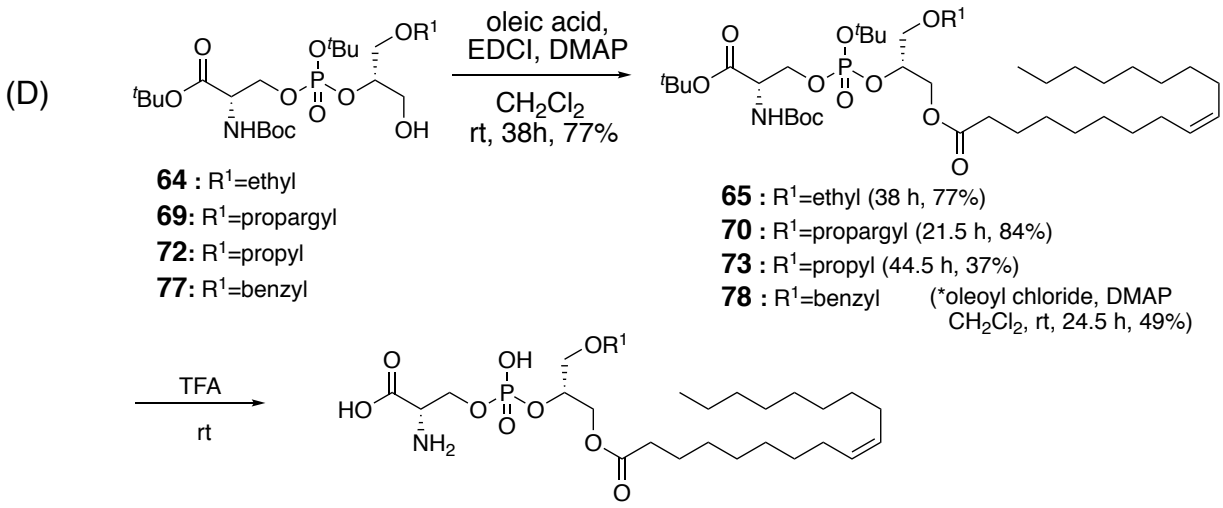


Figure 28. Synthesis of 3-Oleoyl LysoPS Ether Derivatives 6, 7, 8, 9

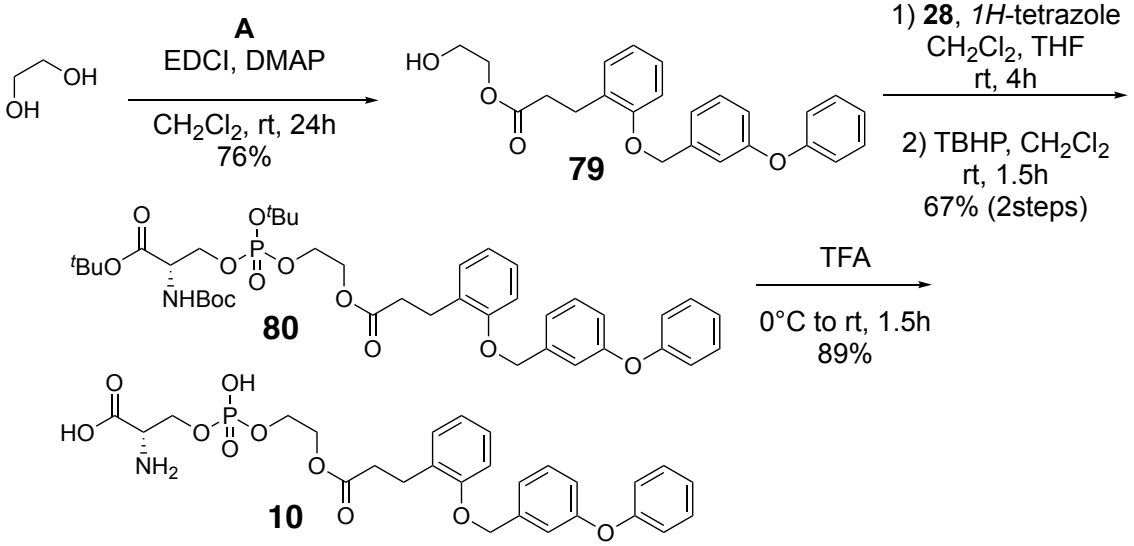


Figure 29. Synthesis of reference compound 10 (*O*-(hydroxy(2-((3-(2-((3-phenoxybenzyl)oxy)phenyl)propanoyl)oxy)ethoxy)phosphoryl)-*L*-serine (deoxy-LysoPS-C2-C3-ph-o-O-Bn-m-O-ph))

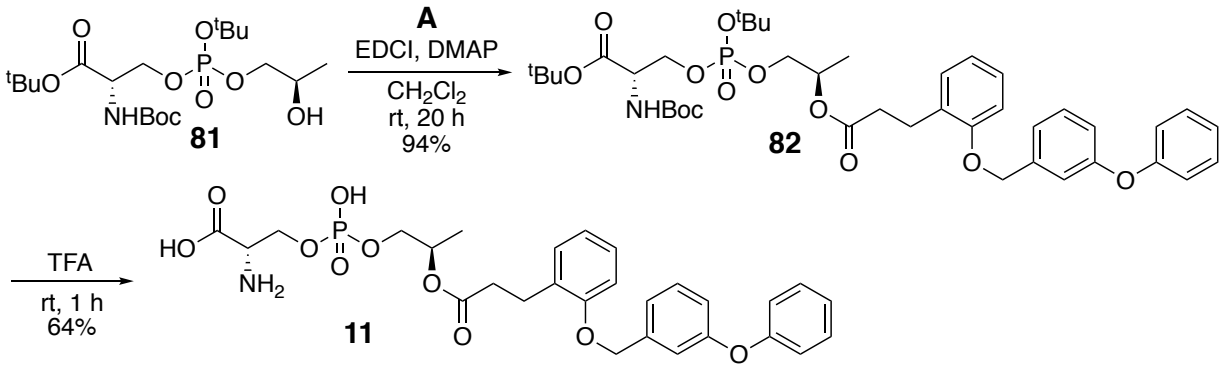


Figure 30. Synthesis of LPS1 selective agonists 11 (1-deoxy-2-acyl o-OBn-m-OPh: *O*-(hydroxy((*R*)-2-((3-(2-((3-phenoxybenzyl)oxy)phenyl)propanoyl)oxy)propoxy)phosphoryl)-*L*-serine)

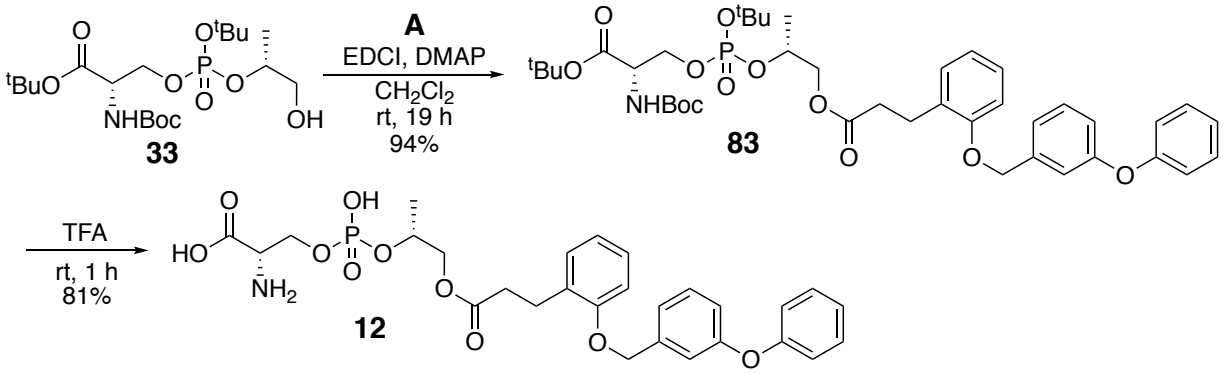


Figure 31. Synthesis of 12 (*O*-(hydroxy(((*R*)-1-((3-(2-((3-phenoxybenzyl)oxy)phenyl)-propanoyl)oxy)propan-2-yl)oxy)phosphoryl)-*L*-serine)

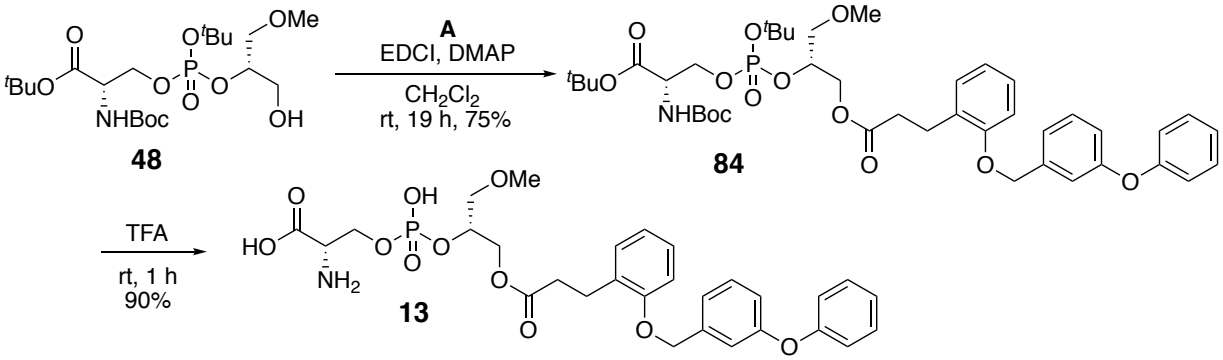


Figure 32. Synthesis of 13 (1-methoxy-3-acyl LysoPS C3-ph-o-O-Bn-m-O-ph: *O*-(hydroxy(((*R*)-1-methoxy-3-((3-(2-((3-phenoxybenzyl)oxy)phenyl)propanoyl)oxy)propan-2-yl)oxy)phosphoryl)-*L*-serine)

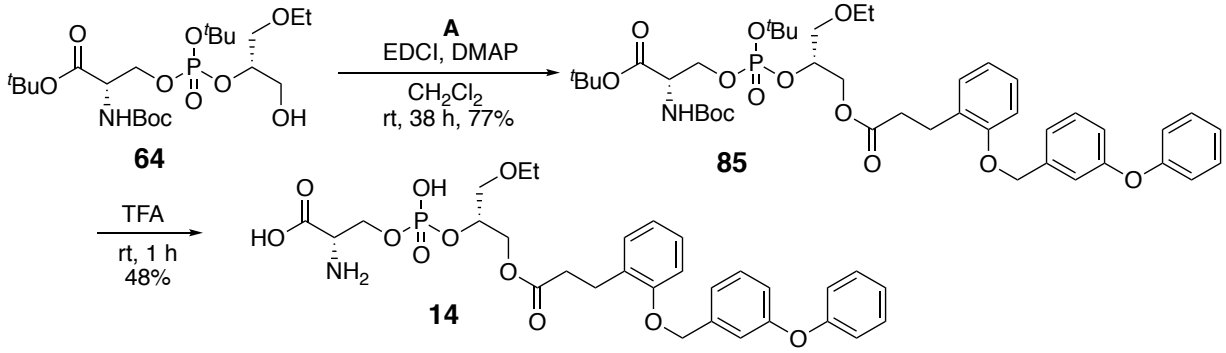


Figure 33 Synthesis of 14 (*O*-((((*R*)-1-ethoxy-3-((3-(2-((3-phenoxybenzyl)oxy)phenyl)-propanoyl)oxy)propan-2-yl)oxy)(hydroxy)phosphoryl)-*L*-serine: 1-ethoxy-3-acyl LysoPS C3-ph-o-O-Bn-m-O-ph)

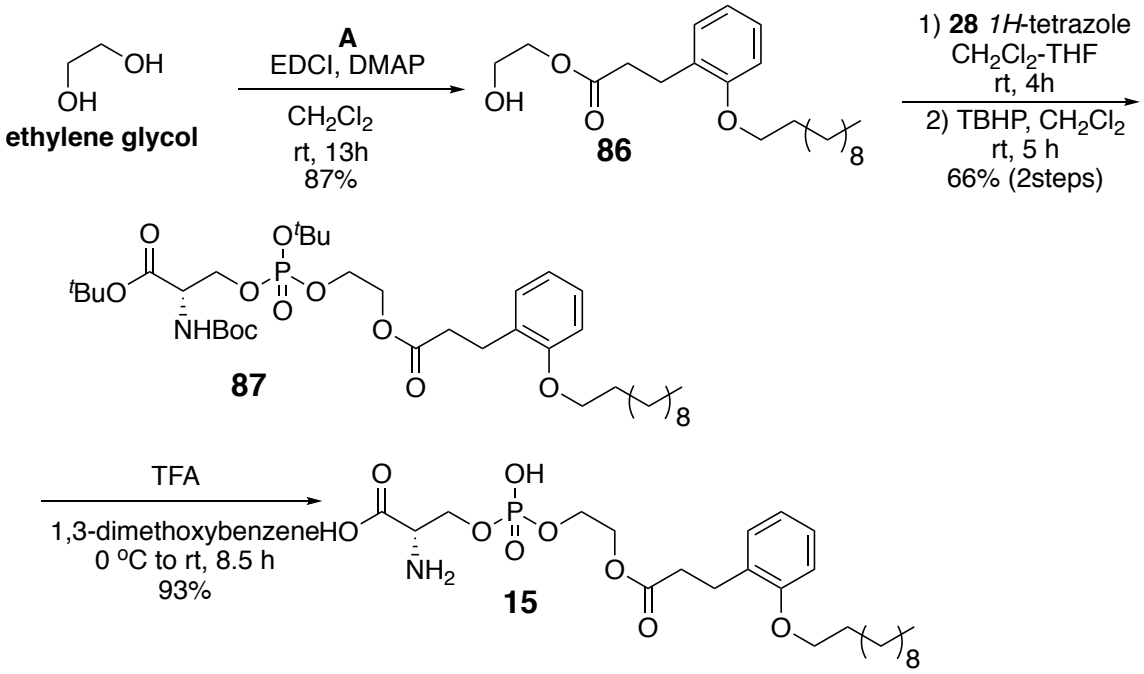


Figure 34. Synthesis of 15 (deoxy-LysoPS C2 C3-ph-o-O-C11: *O*-(hydroxy(2-((3-(2-(undecyloxy)phenyl)propanoyl)oxy)ethoxy)phosphoryl)-*L*-serine)

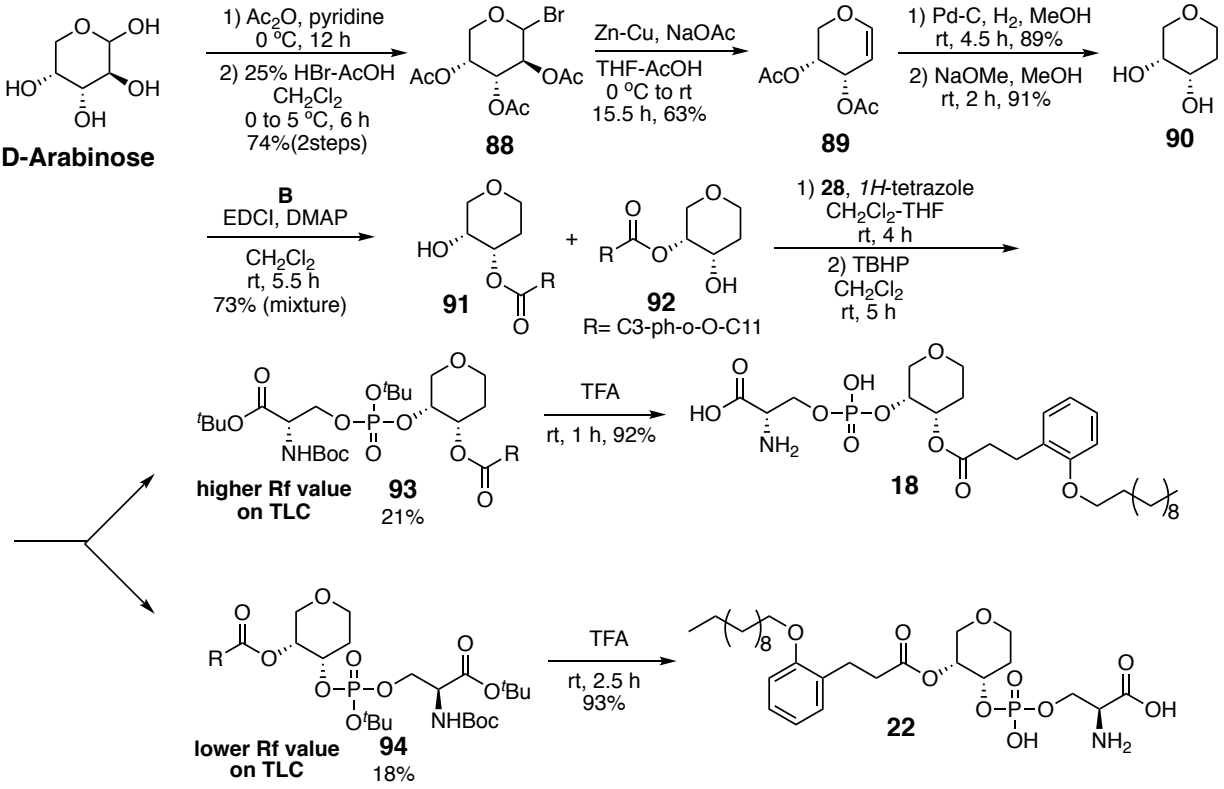


Figure 35. Synthesis of cyclic 18 (*O*-((((3*R*,4*S*)-4-((3-(2-(dodecyloxy)phenyl)propanoyl)oxy)-tetrahydro-2*H*-pyran-3-yl)oxy)(hydroxy)phosphoryl)-*L*-serine) and 22 (*O*-(hydroxy(((3*R*,4*S*)-3-((3-(2-(undecyloxy)phenyl)-propanoyl)oxy)tetrahydro-2*H*-pyran-4-yl)oxy)phosphoryl)-*L*-serine)[18]

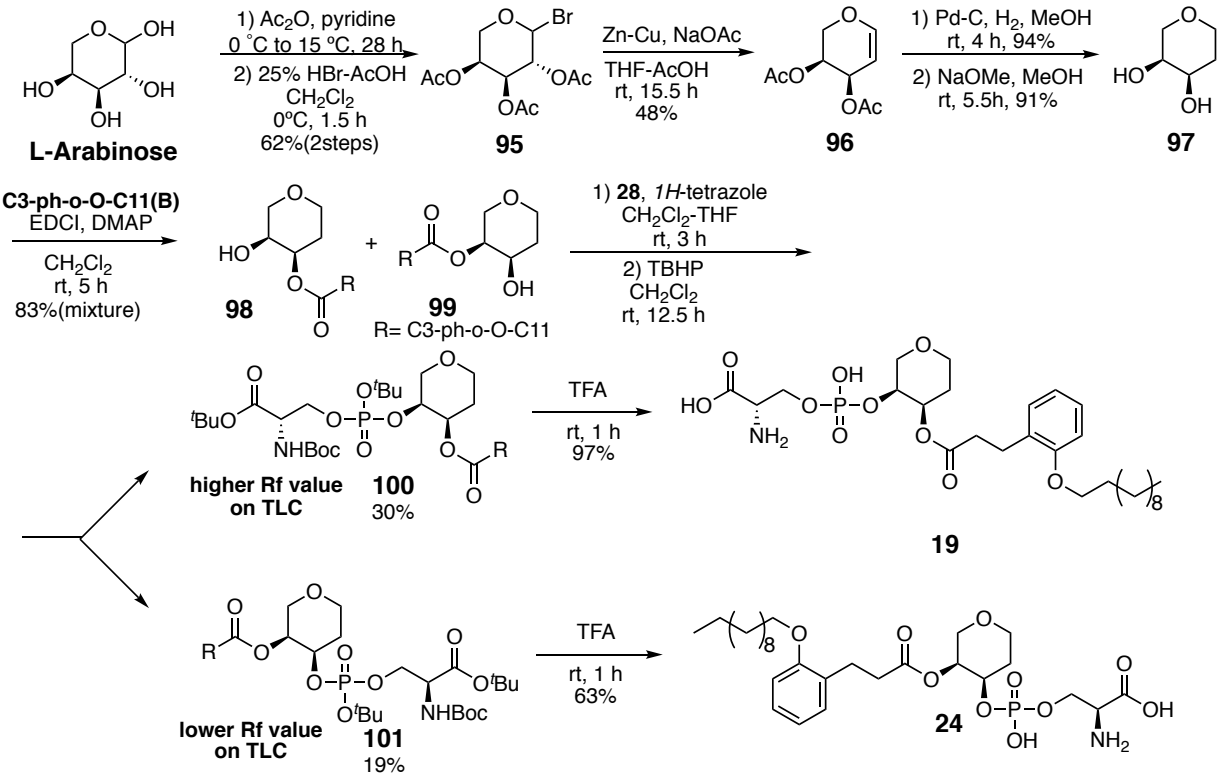


Figure 36. Synthesis of cyclic glycol derivatives 19 (*O*-(hydroxy(((3*S*,4*R*)-4-((3-(2-(undecyloxy)phenyl)propanoyl)oxy)tetrahydro-2*H*-pyran-3-yl)oxy)phosphoryl)-*L*-serine)

and 24 (*O*-(hydroxy(((3*S*,4*R*)-3-((3-(2-(undecyloxy)phenyl)propanoyl)oxy)tetrahydro-2*H*-pyran-4-yl)oxy)phosphoryl)-*L*-serine).

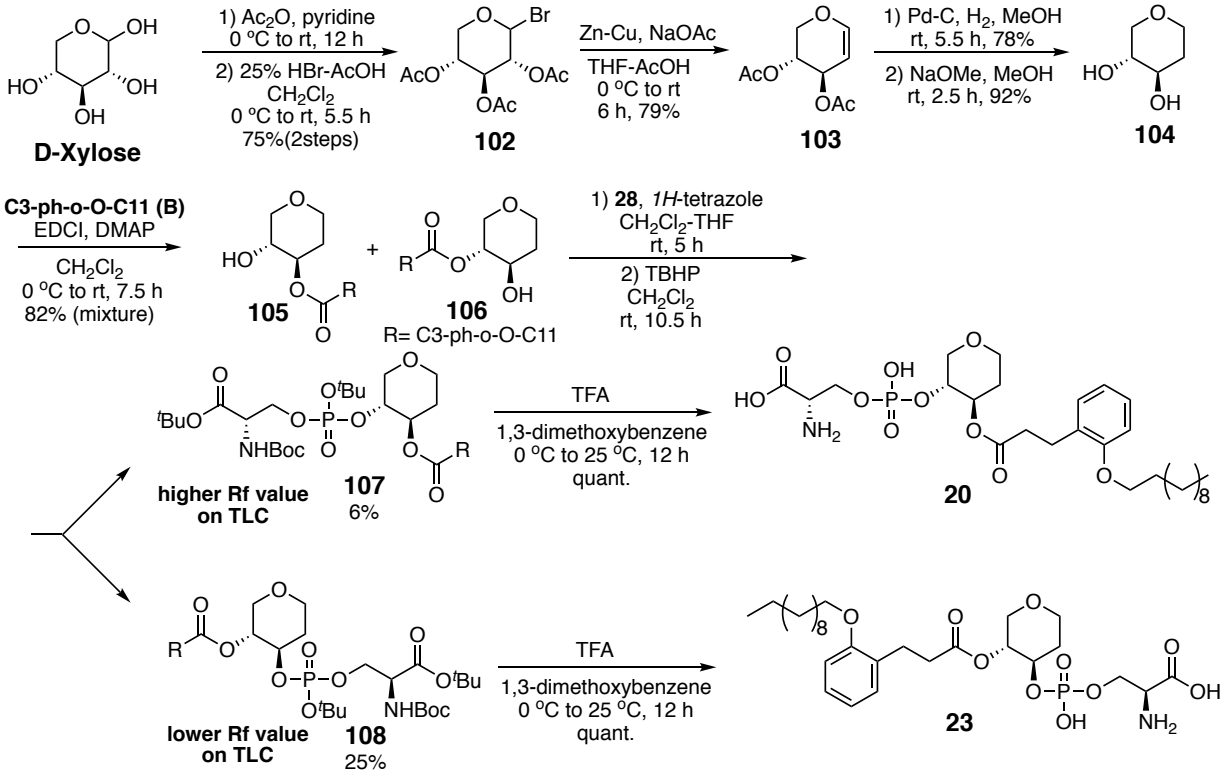


Figure 37. Synthesis of cyclic 20 (*O*-(hydroxy(((3*R*,4*R*)-4-((3-(2-(undecyloxy)phenyl)-propanoyl)oxy)tetrahydro-2*H*-pyran-3-yl)oxy)phosphoryl)-*L*-serine) and 23 (*O*-(hydroxy(((3*R*,4*R*)-3-((3-(2-(undecyloxy)phenyl)propanoyl)oxy)tetrahydro-2*H*-pyran-4-yl)oxy)phosphoryl)-*L*-serine).

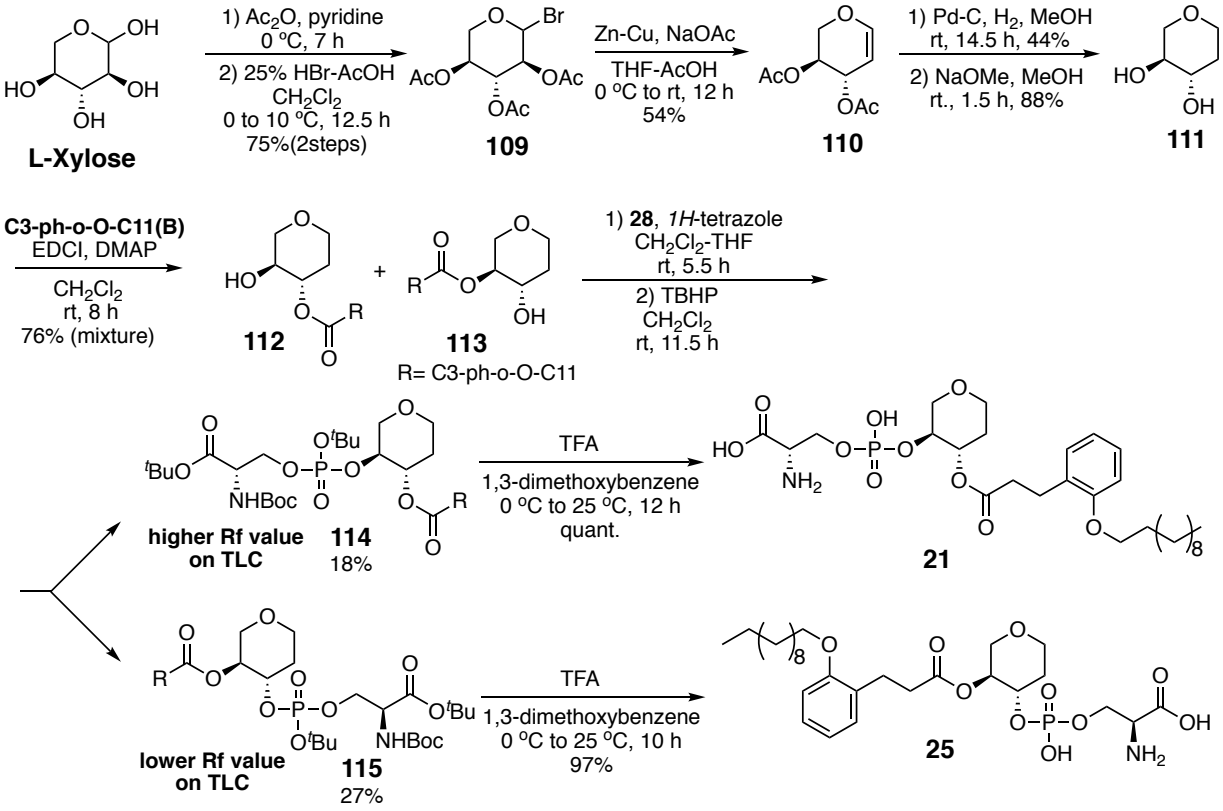


Figure 38. Synthesis of cyclic 21 (*O*-(hydroxy(((3*S*,4*S*)-4-((3-(2-(undecyloxy)phenyl)-propanoyl)oxy)tetrahydro-2*H*-pyran-3-yl)oxy)phosphoryl)-*L*-serine) and 25 (*O*-(hydroxy(((3*S*,4*S*)-3-((3-(2-(undecyloxy)phenyl)propanoyl)oxy)tetrahydro-2*H*-pyran-4-yl)oxy)phosphoryl)-*L*-serine).

Experimental Section

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-, ^{13}C- and ^{31}P-NMR spectra were recorded on a Bruker Avance 400 spectrometer. Chemical shifts of ^{1}H- and ^{13}C-NMR spectra are given in parts per million (ppm), relative to those of chloroform (7.26 ppm for ^{1}H NMR spectra, and 77.00 ppm for ^{13}C NMR spectra) or with tetramethylsilane as an internal standard. Chemical shifts of ^{31}P signals are reported in parts per million (ppm), relative to that of phosphoric acid in water (85% w/w, as 0.00 ppm). Mass spectra were recorded on a Bruker microTOF-05 (ESI-TOF) in the positive and negative ion detection modes. All HPLC separations were carried out using an ODS column, Unison UK-C18 (4.6 x 250 mm, Imtakt, Kyoto, Japan). Eluted products were detected by measuring the UV absorbance at 270 nm. Solvent A was 10% acetonitrile and 0.1% formic acid in water (v/v) and solvent B was 0.1% formic acid in acetonitrile, unless otherwise specified. A flow rate of 1 mL/min was used for all analytical separations. The purity of all the compounds, whose biological activities were studied, is more than 95%.

Synthesis of LysoPS Derivatives

Previously synthesized samples of **1a (deoxy-LysoPS C2 (18:1)**, **1b (deoxy-LysoPS C3 (18:1))** and **1c (deoxy-LysoPS C4 (18:1))** [11, 19] were used for the assay.

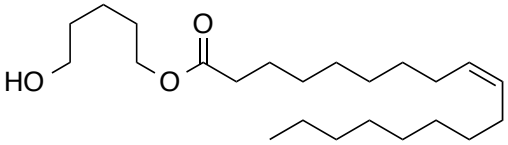
1. Synthesis of deoxy-LysoPS derivatives

1-1. Synthesis of protected *L*-serine 28 (*tert***-Butyl *O*-(*tert*-butoxy(diisopropylamino)phosphaneyl)-*N*-(*tert*-butoxycarbonyl)-*L*-serinate) (Figure 20)**

Protected *L*-serinate **(28)** was synthesized from **27** and bis(diisopropylamino)*tert*-butylphosphine as previously described.[11, 13, 14] **27** was a protected *L*-serine derivatived from **26**.

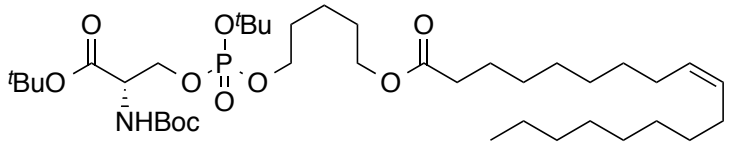
Synthesis of 1d ((*O*-(hydroxy((5-(oleoyloxy)pentyl)oxy)phosphoryl)-*L*-serine: deoxy-LysoPS C5 (18:1)) (Figure 21)

Synthesis of 29



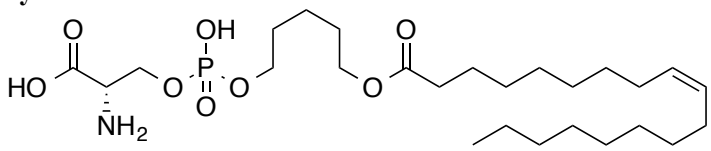
1,5-Pentanediol (420.8m g, 4.040 mmol) and anhydrous pyridine (0.1 mL) were dissolved in anhydrous CH_2Cl_2 (4 mL). Oleoyl chloride (301.7 mg, 1.003 mmol) in CH_2Cl_2 (1.2 mL) was added dropwise to the resulting solution at 0˚C, and the reaction mixture was stirred for 18.5 hours at room temperature. EDCI/HCl (201.1 mg, 1.049 mmol) and DMAP (24.0 mg, 0.196 mmol) were added, and the mixture was stirred for 5 hours. Then 5% aqueous $KHSO_4$ (20 mL) was added, and the resulting mixture was extracted three times with CH_2Cl_2 (20 mL). The combined organic layer was washed twice with water (20 mL) and twice with brine (20 mL). The combined aqueous layer was extracted with CH_2Cl_2 (10 mL). The whole organic solution was dried over Na_2SO_4 and filtered. The filtrate was evaporated and the residue was chromatographed (n-hexane:AcOEt = 3:1) to yield **29** (246.8 mg, 0.670 mmol, 67 %, yellow oil). ^{1}H-NMR ($CDCl_3$): δ = 5.335 (2H, m), 4.068 (2H, t, *J* = 6.6 Hz), 3.645 (2H, t, *J* = 6.6 Hz), 2.280 (2H, t, *J* = 7.6 Hz), 2.000 (4H, m), 1.690-1.559 (6H, m), 1.425 (2H, m), 1.337-1.208 (20H, m), 0.871 (3H, t, *J* = 6.8 Hz). ^{13}C-NMR ($CDCl_3$): δ = 173.95, 129.98, 129.72, 64.13, 62.67, 34.34, 32.27, 31.87, 29.74, 29.66, 29.49, 29.29, 29.14, 29.11, 29.08, 28.45, 27.19, 27.14, 24.97, 22.65, 22.21, 14.07. HRMS (ESI, $[M+Na]^+$): Calcd for $C_{23}H_{44}NaO_3^+$: 391.3183. Found: 391.3169.

Synthesis of 30



Alcohol **29** (168.8 mg, 0.458 mmol) was dissolved in CH_2Cl_2 and co-evaporated with toluene, then **28** (162.0 mg, 0.349 mmol) was added. The mixture was dissolved in CH_2Cl_2 and co-evaporated with toluene. Under an argon atmosphere, the residue was dissolved in anhydrous CH_2Cl_2 (2 mL), and a solution of 1*H*-tetrazole (41.1 mg, 0.587 mmol) in anhydrous THF (2 mL) was added. After a few minutes, a white solid precipitated. The reaction mixture was stirred for 5 h at room temperature, then diluted with H_2O (10 mL) and saturated $NaHCO_3$ aq. (6 ml). The solution was extracted three times with CH_2Cl_2 (10 ml). The combined organic layer was washed with brine (10 mL). The combined aqueous layer was extracted with CH_2Cl_2 (10 mL). Then, the whole organic solution was dried over Na_2SO_4 and filtered. The filtrate was evaporated and the residue was chromatographed (n-hexane: AcOEt: Et_3N = 35:4:1). The eluate was evaporated and the residue was dissolved in anhydrous CH_2Cl_2 (4 mL). A solution of TBHP in decane (5.0-6.0 M) (130.6 μL, 0.653 mmol) was added, and the mixture was stirred for 9 h at room temperature under an argon atmosphere. The solvent was evaporated and the residue was chromatographed (n-hexane : AcOEt = 3:1) to yield **30** (178.2 mg, 0.238 mmol, 68%, thick oil). ^{1}H-NMR ($CDCl_3$): δ = 5.444 (1H, d, *J* = 8.0 Hz), 5.278 (2H, m), 4.283 (2H, m), 4.155 (2H, m), 4.003 (2H, dt *J* = 1.6, 6.6 Hz), 3.935 (2H, m), 2.226 (2H, t, *J* = 7.6 Hz), 1.949 (4H, m), 1.595 (6H, m), 1.418 (29H, m), 1.286-1.196 (20H, m), 0.818 (3H, t, *J* = 6.8 Hz). ^{31}P-NMR ($CDCl_3$): δ = -5.545, -5.674. ^{13}C-NMR ($CDCl_3$): δ = 173.67, 168.24, 155.10, 129.83, 129.58, 83.30, 83.23, 82.46, 82.43, 79.71, 67.23, 67.18, 67.12, 67.06, 63.80, 54.37, 54.29, 34.15, 31.76, 29.73, 29.68, 29.66, 29.64, 29.62, 29.55, 29.37, 29.16, 29.03, 29.00, 28.96, 28.18, 28.06, 27.81, 27.06, 27.02, 24.82, 22.53, 21.93, 13.96. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_{39}H_{74}NNaO_{10}P^+$: 770.4943. Found: 770.4905.

Synthesis of 1d



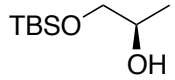
TFA (2.0 mL) was added to **30** (169.8 mg, 0.227 mmol) at 0ºC. The reaction mixture was stirred for 1.5 h at room temperature, then diluted with CH_2Cl_2 (10 mL) and the mixture was evaporated. The residue was chromatographed ($CHCl_3$:MeOH:AcOH = 9:1:0, 8:1:1, 7:1:2 to 6:1:3) to yield **1d** as the AcOH salt (111.5 mg, 0.208 mmol, 92%, white solid). The AcOH salt was dissolved in TFA and evaporated to yield **1d** as the TFA salt (white solid). ^{1}H-NMR ($CDCl_3$): δ = 5.374 (2H, m), 4.644 (2H, m), 4.555 (1H, m), 4.152 (2H, t, *J* = 6.4 Hz), 4.066 (2H, m), 2.395 (2H, t, *J* = 7.6 Hz), 2.005 (4H, m), 1.675 (6H, m), 1.461-1.275 (22H, m), 0.879 (3H, t, *J* = 6.6 Hz). ^{31}P-NMR ($CDCl_3$): δ = -1.228. HRMS (ESI-TOF, $[M-H]^-$): Calcd for $C_{26}H_{49}NO_8P^-$: 534.3201. Found: 534.3185. Mp. 131.0-134.5ºC, colorless cubes. Anal. Calcd. For $C_{26}H_{50}NO_8P \cdot 0.6CF_3COOH$: C, 54.08; H, 8.44; N, 2.32. Found: C, 54.23; H, 8.35; N, 2.27.

Synthesis of C2-LysoPS Derivatives

A previously synthesized sample of ***3R***[19] was used for the present studies.

Synthesis of 2*R* (*O*-(hydroxy(((*R*)-1-(oleoyloxy)propan-2-yl)oxy)phosphoryl)-*L*-serine: 1-deoxy-3-oleoyl-LysoPS (R)) (Figure 22)

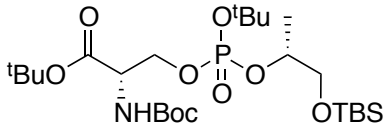
Synthesis of 31



To a solution of (*R*)-1,2-propandiol (201.1 mg, 2.643 mmol) and imidazole (269.9 mg, 3.964 mmol) in anhydrous DMF (4 mL) was added a solution of TBSCl (436.0 mg, 2.893 mmol) in

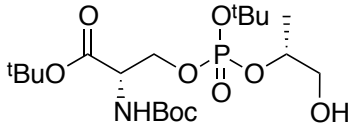
DMF (2 mL) at room temperature. The mixture was stirred for 11 h, then diluted with water (10 mL) and AcOEt (10 mL). The aqueous layer was separated and extracted three times with AcOEt (10 mL x 3). The combined organic layer was dried over Na_2SO_4 and evaporated. The residue was chromatographed (n-hexane:AcOEt=5:1) to yield **31** (478.0 mg, 2.511 mmol, 95 %, colorless oil). ^{1}H-NMR ($CDCl_3$): δ = 3.812 (1H, m), 3.587 (2H, d, *J* = 9.92, 3.36 Hz), 3.587 (2H, d, *J* = 9.92, 3.72 Hz), 2.323 (1H, brs), 1.111 (3H, d, *J* = 8.32 Hz), 0.903 (9H, s), 0.070 (6H, s). ^{13}C NMR ($CDCl_3$): δ = 68.53, 67.93, 25.87, 18.28, 18.18, -5.37, -5.41. HRMS (ESI, $[M+Na]^+$): Calcd for $C_{33}H_{70}NaO_3Si_2^+$: 593.4756. Found: 593.4758.

Synthesis of 32



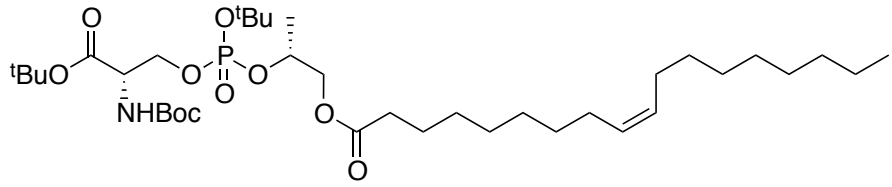
In order to eliminate water, phosphoamidite **28** (202.1 mg, 0.435 mmol) was dissolved in CH_2Cl_2 (5 mL) and toluene (0.5 mL), and the solvent was evaporated under vacuum. To the residue, alcohol **31** (100.1 mg, 0.526 mmol), CH_2Cl_2 (5 mL) and toluene (0.5 mL) were added, and the mixture was evaporated under vacuum. In an argon atmosphere, the resulting residue was dissolved in CH_2Cl_2 (2 mL), and the solution of 1*H*-tetrazole (92.3 mg, 1.317 mmol) in THF (2 mL) was added at room temperature. After a few minutes, a white solid precipitated. The reaction mixture was stirred for 5 h at room temperature, and a solution of *tert*-butylhydrogen peroxide (TBHP) in decane (5.0-6.0 M) (0.175 mL, 0.875 mmol) was added at room temperature. The whole was stirred for 2 hours at room temperature. The solution was diluted with water (10 mL) and the whole was extracted three times with CH_2Cl_2 (10 mL x 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and evaporated. The residue was column-chromatographed (n-hexane:ethyl acetate = 7:2) to yield **32** (226.3 mg, 0.397 mmol, 91%, colorless oil). ^{1}H-NMR ($CDCl_3$): δ = 5.490 (1H, m), 4.407 (1H, m), 4.335 (2H, m), 4.202 (1H, m), 3.687 (1H, m), 3.538 (1H, m), 1.482 (9H, m), 1.466 (9H, s), 1.436 (9H, s), 1.300 (3H, dd, *J* = 10.60, 6.32 Hz), 0.883 (9/2H, s), 0.880 (9/2H, s), 0.057 (3/2H, s), 0.052 (3/2H, s), 0.048 (3/2H, s), 0.045 (3/2H, s). ^{31}P-NMR ($CDCl_3$): δ = -6.034, -6.354. ^{13}C-NMR ($CDCl_3$): δ = 168.41, 155.25, 83.44, 83.37, 83.31, 82.54, 82.51, 79.80, 75.48, 75.44, 75.42, 75.38, 67.30, 67.25, 66.47, 66,44, 66.40, 66.37, 54.48, 54.40, 29.83, 29.81, 29.79, 29.77, 28.31, 27.95, 27.94, 25.83, 18.27, 18.13, 18.09, 18.05, -5.38, -5.41, -5.42, -5.46. HRMS (ESI, $[M+Na]^+$): Calcd for $C_{25}H_{52}NNaO_9PSi^+$: 592.3041. Found: 592.3051.

Synthesis of 33



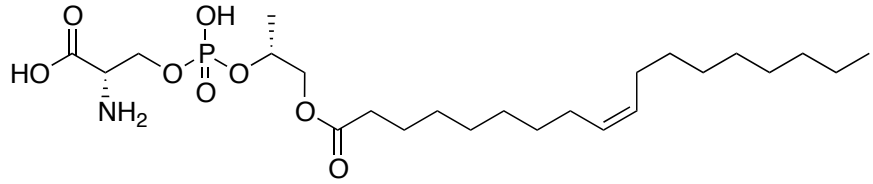
To a solution of **32** in THF (3 mL) and pyridine (150 μL) was added dropwise HF•pyridine (150 μL). The mixture was stirred for 20 h, then diluted with aqueous 5% $KHSO_4$ (10 mL) and AcOEt (10 mL). The aqueous layer was separated and extracted three times with AcOEt (10 mL x 3). The combined organic layer was dried over Na_2SO_4 and the solvent was evaporated. The residue was chromatographed (n-hexane:AcOEt = 1:1 to 0:1) to yield **33** (129.0 mg, 0.283 mmol, 73 %, white solid). ^{1}H-NMR ($CDCl_3$): δ = 5.516 (1H, m), 4.533 (1H, m), 4.386-4.218 (3H, m), 3.633 (2H, m), 2.637 (1H, brs), 1.512-1.481 (18H, m), 1.450 (9H, s), 1.286 (3H, t, *J* = 6.04 Hz). ^{31}P-NMR ($CDCl_3$): δ = -4.990, -5.084. ^{13}C-NMR ($CDCl_3$): δ = 168.51, 168.39, 155.25, 82.87, 80.03, 67.61, 67.06, 66.91, 54.50, 54.42, 29.82, 29.80, 29.78, 29.76, 28.32, 27.96, 17.89, 17.83, 17.76. HRMS (ESI, $[M+Na]^+$): Calcd for $C_{19}H_{38}NNaO_9P^+$: 478.2176. Found: 478.2187. Mp: 90.0-90.5 ˚C.

Synthesis of 34



To a solution of **33** (34.8 mg, 0.0764 mmol) and 4-dimethylaminopyridine (DMAP) (19.1 mg, 0.159 mmol) in CH_2Cl_2 (0.5 mL) was added a solution of oleoyl chloride (30.5 mg, 0.101 mmol) in CH_2Cl_2 (1 mL). The mixture was stirred for 18 h at room temperature, then MeOH (1 mL) and EDCI (34.0 mg, 0.178 mmol) were added and stirring was continued for 3 h at room temperature. The reaction mixture was evaporated and the residue was column-chromatographed (n-hexane: ethyl acetate = 4:1) to yield **34** (43.6 mg, 0.0606 mmol, 79%, colorless oil). ^{1}H-NMR ($CDCl_3$): δ = 5.503 (1H, m), 5.340 (2H, m), 4.634 (1H, m), 4.352 (2H, m), 4.228 (1H, m), 4.109 (2H, m), 2.334 (2H, m), 2.006 (4H, m), 1.613 (2H, m), 1.496-1.476 (18H, m), 1.444 (9H, s), 1.351-1.253 (23H, m), 0.878 (3H, t, *J* = 6.82 Hz). ^{31}P-NMR ($CDCl_3$): δ = -6.086, -6.476. ^{13}C-NMR ($CDCl_3$): δ = 173.41, 173.38, 168.37, 155.24, 129.99, 129.73, 83.76, 82.64, 82.61, 79.89, 77.32, 77.00, 76.68, 72.76, 72.70, 72.66, 72.60, 67.42, 66.70, 66.64, 54.47, 54.39, 34.09, 34.03, 31.89, 29.83, 29.79, 29.76, 29.71, 29.51, 29.31, 29.30, 29.20, 29.18, 29.12, 28.32, 27.97, 27.95, 27.21, 27.17, 24.83, 22.67, 18.16, 18.12, 18.09, 14.10. HRMS (ESI, $[M+Na]^+$): Calcd for $C_{37}H_{70}NNaO_{10}P^+$: 742.4630. Found: 742.4614.

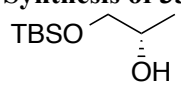
Synthesis of 2*R*



Protected LysoPS derivative **34** (42.1 mg, 0.0585 mmol) was dissolved in TFA (1.0 mL) and the solution was stirred at room temperature for 1 hour, then evaporated. The residue was chromatographed ($CHCl_3$:MeOH:AcOH = 8:1:1) to yield ***2R*** as the AcOH salt (26.9 mg, 0.0530 mmol, 91%, white powder). The AcOH salt was dissolved in TFA and evaporated to yield ***2R*** as the TFA salt (white powder). ^{1}H-NMR ($CDCl_3$/TFA-d = 4:1): δ = 5.365 (2H, m), 4.665 (3H, m), 4.552 (1H, m), 4.284 (1H, m), 4.176 (1H, m), 2.419 (2H, m), 2.018 (4H, m), 1.620 (2H, m), 1.379-1.271 (23H, m), 0.875 (3H, t, *J* = 6.66 Hz). ^{31}P-NMR ($CDCl_3$/TFA-d = 4:1): δ = -2.252. HRMS (ESI, $[M-H]^-$): Calcd. for $C_{24}H_{45}NO_8P^-$: 506.2888. Found: 506.2904. Mp: 151.0-152.0 ºC. Anal. Calcd. For $C_{26}H_{42}F_2NO_9P$•0.5CF_3COOH: C, 53.18; H, 8.30; N, 2.48. Found: C, 53.31; H, 8.30; N, 2.53.

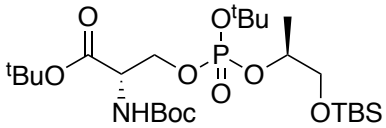
Synthesis of 2*S* (*O*-(hydroxy(((*S*)-1-(oleoyloxy)propan-2-yl)oxy)phosphoryl)-*L*-serine (1-deoxy-3-oleoyl-LysoPS (S)) (Figure 23)

Synthesis of 35



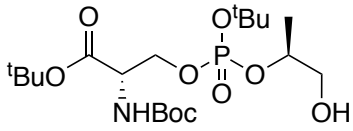
35 was synthesized from (S)-(+)-1,2-propanediol in 64 % yield in a smilar manner to **31**. **35:** Colorless oil. ^{1}H-NMR ($CDCl_3$): δ = 3.799 (1H, m), 3.572 (1H, dd, *J* = 3.4, 9.8 Hz), 3.336 (1H, dd, *J* = 7.8, 9.8 Hz), 1.099 (3H, d, *J* = 6.4 Hz), 0.892 (9H, s), 0.09 (6H, s). ^{13}C-NMR ($CDCl_3$): δ = 68.52, 67.90, 25.84, 18.25, 18.18, -5.40, -5.44. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_9H_{22}NaO_2Si^+$: 213.1281. Found: 213.1299.

Synthesis of 36



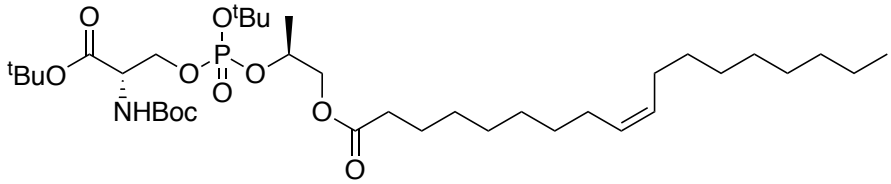
36 was synthezied from **28** and **35** in 76 % yield in a similar manner to **32**. **36**: Thick oil. ^{1}H-NMR ($CDCl_3$): δ = 5.505 (1H, dd, *J* = 8.2, 14.2 Hz), 4.413 (1H, m), 4.328 (2H, m), 4.247-4.159 (1H, m), 3.681 (1H, dd, *J* = 5.2, 10.4 Hz), 3.534 (1H, m), 1.479-1.433 (27H, m), 1.300 (3H, dd, *J* = 6.0 Hz), 0.879 (9H, s), 0.049 (6H, s). ^{31}P-NMR ($CDCl_3$): δ = -6.15. ^{13}C-NMR ($CDCl_3$): δ = 168.46, 168.42, 168.39, 155.28, 83.42, 83.37, 83.34, 83.29, 82.52, 82.48, 79.79, 79.76, 75.54, 75.47, 75.41, 67.35, 67.31, 67.29, 67.23, 66.42, 66.40, 66.36, 66.33, 54.50, 54.47, 54.42, 54.38, 31.55, 29.81, 29.80, 29.77, 28.30, 27.94, 25.82, 18.26, 18.14, 18.11, -5.41, -5.44. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_{25}H_{52}NNaO_9PSi^+$: 592.3041. Found: 592.3015.

Synthesis of 37



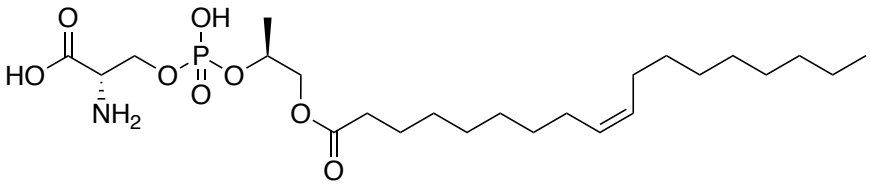
37 was synthesized from **36** in 79% yield in a similar manner to **33**. **37**: White solid. ^{1}H-NMR ($CDCl_3$): δ = 5.522 (1H, m), 4.498 (1H, m), 4.328 (2H, m), 4.245 (1H, m), 3.617 (2H, m), 2.987 (1H, brs), 1.490 (9H, m), 1.470 (9H, s), 1.437 (9H, s), 1.281 (3H, m). ^{31}P-NMR ($CDCl_3$): δ = -5.25, -5.28. ^{13}C-NMR ($CDCl_3$): δ = 168.49, 168.47, 168.44, 168.42, 155.25, 84.22, 84.15, 83.97, 83.89, 82.81, 82.75, 79.98, 77.45, 77.42, 77.39, 77.23, 67.59, 67.55, 66.94, 66.90, 66.81, 66.77, 54.47, 54.39, 29.78, 29.74, 28.29, 27.94, 17.87, 17.81, 17.80, 17.74. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_{19}H_{38}NNaO_9PSi^+$: 478.2176. Found: 478.2181. Mp. 114.2°C-115.0°C, white solid.

Synthesis of 38



38 was synthesized from **37** and oleoyl chloride in 85 % yield in a similar manner to **34**. **38**: Thick oil. ^{1}H-NMR ($CDCl_3$): δ = 5.513 (1H, m), 5.334 (2H, m), 4.629 (1H, m), 4.332 (2H, m), 4.223 (1H, m), 4.153-4.045 (2H, m), 2.335 (2H, m), 2.008 (4H, m), 1.600 (2H, m), 1.470 (27H, m), 0.871 (3H, t, *J* = 6.8 Hz). ^{31}P-NMR ($CDCl_3$): δ = -6.28, -6.37. ^{13}C-NMR ($CDCl_3$): δ = 173.38, 173.37,168.38, 168.34, 155.24, 155.22, 129.97, 129.70, 83.30, 83.76, 83.72, 83.69, 82.60, 82.59, 79.86, 72.86, 72.80, 72.69, 72.63, 67.46, 67.44, 67.40, 67.34, 66.67, 66.64, 66.60, 66.57, 54.49, 54.43, 54.42, 54.40, 54.35, 34.06, 34.00, 31.87, 29.79, 29.74, 29.68, 29.62, 29.49, 29.33, 29.29, 29.18, 29.17, 29.10, 28.30, 27.94, 27.19, 27.15, 24.81, 22.65, 18.19, 18.15, 18.11, 14.08. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_{37}H_{70}NNaO_{10}P^+$: 742.4630. Found: 742.4670. Anal. Calcd. For $C_{37}H_{70}NO_{10}P$: C, 61.73; H, 9.80; N, 1.95. Found: C, 61.54; H, 9.50; N, 1.93.

Synthesis of 2*S*

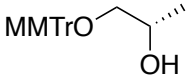


2*S* was synthesized from **38** in 73 % yield in a similar manner to **2*R***. **2*S*** :the TFA salt (yellow solid). ^{1}H-NMR ($CDCl_3$): δ = 5.354 (2H, m), 4.550 (4H, m), 4.273 (1H, m), 4.143 (1H, m), 2.403 (2H, t, *J* = 7 Hz), 1.998 (4H, m), 1.686 (2H, m), 1.343-1.270 (23H, m), 0.875 (3H, t, *J* = 6.6 Hz). ^{31}P-NMR ($CDCl_3$): δ = -2.55. HRMS (ESI-TOF, $[M-H]^-$): Calcd for $C_{25}H_{45}NO_8P^-$: 506.2888.

Found: 506.2931. Mp: 133.5°C -134.5°C, colorless solid. Anal. Calcd. for $C_{24}H_{46}NO_8P \cdot 0.6CF_3COOH$: C, 52.55; H, 8.15; N, 2.43. Found: C, 52.44; H, 7.92; N, 2.17.

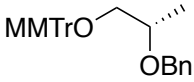
Synthesis of 3*S* (*O*-(hydroxy((*S*)-2-(oleoyloxy)propoxy)phosphoryl)-*L*-serine (1-deoxy-2-oleoyl LysoPS) (Figure 24)

Synthesis of 39



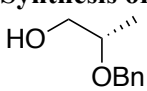
(S)-(+)-1,2-Propanediol (198.0 mg, 2.602 mmol) and DIPEA (1.358 mL, 7.796 mmol) were dissolved in anhydrous CH_2Cl_2 (9 ml), and the solution was cooled to 0°C. 4-Methoxytriphenylmethyl chloride (876.1 mg, 2.837 mmol) was added, and the mixture was stirred at room temperature for 3.5 h under an argon atmosphere. H_2O (10 mL) was added, and the whole was extracted three times with CH_2Cl_2 (10 mL). The organic solution was washed with brine (10 mL). The aqueous layer was extracted three times with CH_2Cl_2 (10 mL). The combined organic layer was dried over Na_2SO_4 and filtered. The filtrate was evaporated and the residue was chromatographed (n-hexane:AcOEt = 8:1 to 4:1) to yield **39** (colorless oil, 839.3 mg, 2.409 mmol, 93%). ^{1}H-NMR ($CDCl_3$): δ = 7.435 (4H, m), 7.330-7.273 (6H, m), 7.227 (2H, m), 3.972 (1H, m), 3.798 (3H, s), 3.129 (1H, dd, *J* = 3.2, 9.2 Hz), 2.989 (1H, dd, *J* = 7.8, 9.4 Hz), 2.346 (1H, brs), 1.102 (3H, d, *J* = 6.4 Hz). ^{13}C-NMR ($CDCl_3$): δ = 158.57, 144.38, 144.34, 135.53, 130.33, 128.36, 127.82, 126.92, 113.11, 86.31, 68.88, 67.06, 55.19, 18.96. HRMS (ESI, $[M+Na]^+$): Calcd for $C_{23}H_{24}NaO_3^+$: 371.1618 Found: 371.1622.

Synthesis of 40



Alcohol **39** (404.0 mg, 1.159 mmol) was dissolved in anhydrous DMF (6 ml) and the solution was cooled to 0°C. NaH (191.1 mg, 4.778 mmol) was added, and the mixture was stirred for 40 min under an argon atmosphere. A solution of benzyl bromide (546 μL, 4.590 mmol) in anhydrous DMF (2 mL) was added dropwise and stirring was continued for 3.5 h at room temperature. Benzyl bromide (138 μL, 1.160 mmol) was added and stirring was continued for 1 h. NaH (88.7 mg, 2.218 mol) was added and the mixture was further stirred for 40 min at 0 °C, and for 2 h at room temperature. AcOEt (10 mL) and H_2O (10 mL) were added and the organic layer was separated. The aqueous layer was extracted three times with AcOEt (10 mL). The combined organic layer was washed with brine. The combined aqueous layer was extracted with AcOEt (10 mL). The whole organic solution was dried over Na_2SO_4 and filtered. The filtrate was evaporated and the residue was chromatographed (n-hexane:Et_2O = 30:1 to 10:1) to yield **40** (colorless oil, 391.6 mg, 0.893 mmol, 77%). ^{1}H-NMR ($CDCl_3$): δ = 7.473 (4H, m), 7.380-7.271 (11H, m), 7.208 (2H, m), 6.814 (2H, m), 4.616 (2H, d, *J* = 3.2 Hz), 3.790 (3H, s), 3.714 (1H, m), 3.244 (1H, dd, *J* = 6.0, 9.6 Hz), 3.030 (1H, dd, *J* = 4.4, 9.6 Hz), 1.185 (3H, d, *J* = 6.4 Hz). ^{13}C-NMR ($CDCl_3$): δ = 158.45, 144.71, 144.64, 139.04, 135.88, 130.39, 128.49, 128.28, 127.71, 127.59, 127.35, 126.74, 113.00, 86.19, 74.43, 71.21, 67.52, 55.17, 17.64. HRMS (ESI, $[M+Na]^+$): Calcd for $C_{30}H_{30}NaO_3^+$: 461.2087 Found: 461.2103.

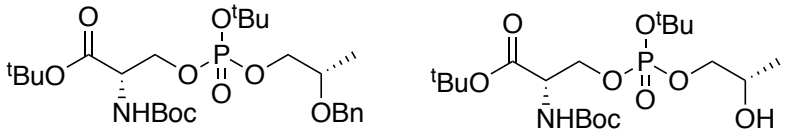
Synthesis of 41



40 (365.8 mg, 0.834 mmol) was dissolved in MeOH (1 mL) and CH_2Cl_2 (3 mL). CSA (19.6 mg, 0.084 mmol) was added, and the mixture was stirred for 6 h at room temperature. The solvent was evaporated and then MeOH (1.0 mL) and CH_2Cl_2 (2.4 mL) were added. Next, CSA (41.0 mg, 0.176 mmol) was added, and stirring was continued for 3 h. CH_2Cl_2 (4 mL) was added and the mixture was evaporated. The residue was chromatographed (n-hexane:AcOEt = 4:1 to 2:1) to yield **41** (colorless oil, 94.3 mg, 0.567 mmol, 68%). ^{1}H-NMR ($CDCl_3$): δ = 7.360-7.272 (5H, m),

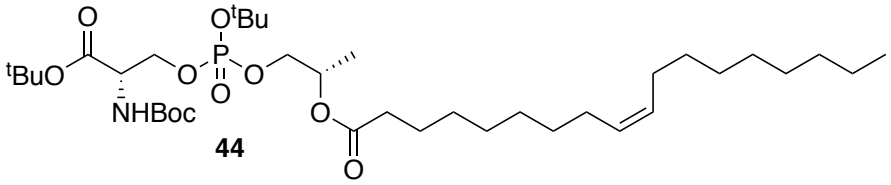
4.661 (1H, d, J = 11.6 Hz), 4.494 (1H, d, J = 11.6 Hz), 3.688 (1H, m), 3.621 (1H, dd, 3.4, 11.4 Hz), 3.507 (1H, dd, J = 7.0, 11.4 Hz), 1.921 (1H, brs), 1.185 (3H, d, J = 6.0 Hz). ^{13}C-NMR ($CDCl_3$): δ = 138.43, 128.47, 127.72, 75.52, 70.80, 66.38, 15.84. HRMS (ESI, $[M+Na]^+$): Calcd for $C_{10}H_{14}NaO_2^+$: 189.0886 Found: 189.0924.

Synthesis of 42 and 43



28 (197.7 mg, 0426 mmol) was dissolved in CH_2Cl_2 and co-evaporated with toluene. Alcohol **41** (93.9 mg, 0.565 mmol) was added to the residue, and the mixture was dissolved in CH_2Cl_2 and co-evaporated with toluene. Under an argon atmosphere, the mixture was dissolved in anhydrous CH_2Cl_2 (2 mL), and a solution of 1*H*-tetrazole (48.5 mg, 0.692 mmol) in anhydrous THF (2 mL) was added at room temperature. After a few minutes, a white solid precipitated. The reaction mixture was stirred for 8 h at room temperature. TBHP in decane (5.0-6.0 M) (0.179 mL, 0.895 mmol) was added, and stirring was continued for 10.5 h at room temperature. The solution was diluted with water (10 mL) and extracted three times with CH_2Cl_2 (10 mL). The combined organic layer was washed with brine (10 mL). The combined aqueous layer was extracted with CH_2Cl_2 (10 mL). The whole organic solution was dried over Na_2SO_4 and filtered. The filtrate was evaporated and the residue was chromatographed (n-hexane:AcOEt = 2:1). The mixture was chromatographed (n-hexane:AcOEt = 3:2) to yield crude **42** (149.4 mg). The crude mixture (38.3 mg) was dissolved in MeOH (2 mL), and Pd-C (10%, 6.8 mg) was added. The mixture was stirred for 5.5 h under an H_2 atmosphere. AcOH (0.5 mL) was added. Stirring was continued for 14.5 h, then the mixture was filtered. H_2O (10 mL) was added to the filtrate and the mixture was extracted three times with AcOEt (10 mL). The combined organic layer was washed with brine. The combined aqueous layer was extracted with AcOEt(10 mL). The whole organic solution was dried over Na_2SO_4 and filtered. The filtrate was evaporated and the residue was chromatographed to yield **43** (14.6 mg, 0.033 mmol, 30 %, two steps, white solid). ^{1}H-NMR ($CDCl_3$): δ = 5.502 (1H, dd, J = 7.6, 20.8 Hz), 4.385-4.223 (3H, m), 4.005 (2H, m), 3.807 (1H, m), 1.504-1.446 (27H, m), 1.175 (3H, dd, J = 2.2, 6.6 Hz). ^{31}P-NMR ($CDCl_3$): δ = -5.00. ^{13}C-NMR ($CDCl_3$): δ = 168.45, 168.39, 155.23, 84.17, 84.15, 84.10, 84.08, 82.86, 82.85, 80.07, 72.93, 72.87, 72.80, 67.63, 67.59, 67.55, 66.62, 66.56, 54.48, 54.41, 29.79, 29.75, 28.30, 27.95, 18.39, 18.37. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_{19}H_{38}NNaO_9P^+$: 478.2176. Found: 478.2169. Mp: 103.5-104.0°C.

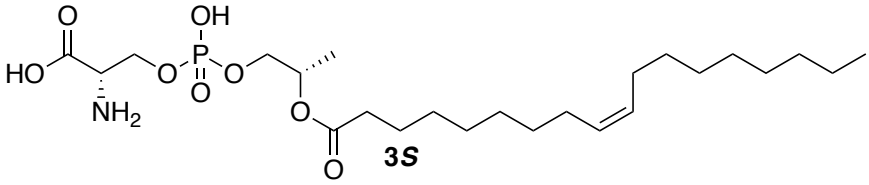
Synthesis of 44



44

44 was synthesized from **43** and oleoyl chloride in 74 % yield in a similar manner to **34**. **44:** Colorless oil. ^{1}H-NMR ($CDCl_3$): δ = 5.478 (1H, t, J = 7.4 Hz), 5.324 (2H, m), 5.068 (1H, m), 4.328 (2H, m), 4.205 (1H, m), 3.698 (2H, m), 2.279 (2H, dt, J=2.8, 7.6Hz), 1.991 (4H, m), 1.598 (2H, quintet, J = 7.2 Hz), 1.480-1.432 (27H, m), 1.285-1.220 (23H, m), 0.862 (3H, t, J = 6.8 Hz). ^{13}C-NMR ($CDCl_3$): δ = 173.04, 173.01, 168.28, 155.21, 129.94, 129.69, 83.92, 83.86, 83.80, 82.66, 82.62, 79.88, 68.76, 68.70, 68.51, 68.46, 68.43, 68.40, 67.55, 67.52, 67.47, 67.42, 67.40, 54.43, 54.36, 34.34, 31.85, 29.75, 29.74, 29.72, 29.69, 29.67, 29.47, 29.27, 29.15, 29.08, 29.06, 28.28, 27.92, 27.17, 27.13, 24.83, 22.63, 16.18, 14.06. ^{31}P-NMR ($CDCl_3$): δ = -5.72, -5.83. HRMS (ESI, $[M+Na]^+$): Calcd for $C_{37}H_{70}NNaO_{10}PSi^+$: 742.4630 Found: 742.4619.

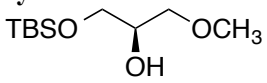
Synthesis of 3*S*



3*S* was synthesized from **44** in 76 % yield in a similar manner to **2*R***. **3*S***: White solid (the TFA salt). ^{1}H-NMR ($CDCl_3$): δ = 5.355 (2H, m), 5.224 (1H, brs), 4.575 (3H, m), 4.035 (2H, brs), 2.382 (2H, m), 2.005 (4H, m), 1.656 (2H, m), 1.302-1.274 (23H, m), 0.877 (3H, t, *J* = 6.8 Hz). ^{31}P-NMR ($CDCl_3$): δ = -1.66. HRMS (ESI-TOF, $[M-H]^-$): Calcd. for $C_{24}H_{45}NO_8P^-$: 506.2888. Found: 506.2934. Mp: 137.5°C-140.5°C, colorless solid. Anal. Calcd. for $C_{24}H_{46}NO_8P \cdot 0.8CF_3COOH$: C, 51.35; H, 7.88; N, 2.34. Found: C, 51.30; H, 7.72; N, 2.29.

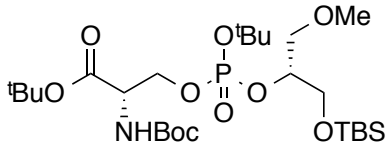
Synthesis of 4*R* (*O*-(hydroxy(((*R*)-1-methoxy-3-(oleoyloxy)propan-2-yl)oxy)- phosphoryl)-*L*-serine (1-methoxy-3-oleoyl LysoPS) (Figure 25)

Synthesis of 46



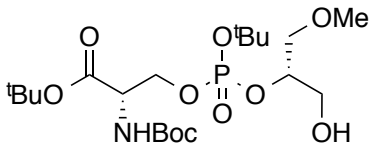
46 was synthesized from the diol **45** in 75 % yield in a similar manner to **31**. **46**: Colorless oil. ^{1}H-NMR ($CDCl_3$): δ = 3.810 (1H, m), 3.635 (2H, m), 3.428 (2H, m), 3.377 (3H, s), 0.898 (9H, s), 0.070 (6H, s). ^{13}C-NMR ($CDCl_3$): δ = 73.45, 70.56, 64.03, 59.16, 25.85, 18.27, -5.45. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_{10}H_{24}NaO_3Si^+$: 243.1387. Found: 243.1391.

Synthesis of 47



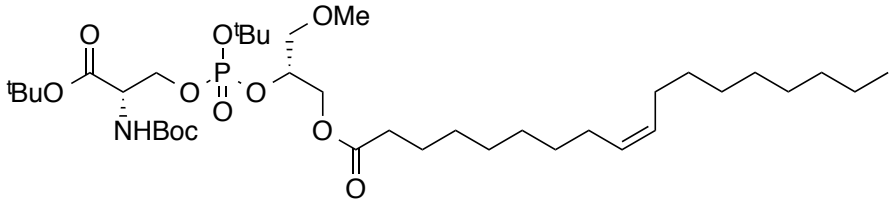
47 was synthesized from **28** and the alcohol **46** in 68 % yield in a similar manner to **32**. **47:** Colorless oil. ^{1}H-NMR ($CDCl_3$): δ = 5.626 (1H, m), 4.471-4.313 (3H, m), 4.241-4.200 (1H, m), 3.635-3.522 (2H, m), 3.738 (2H, m), 3.366 (3H, d, *J* = 2.0 Hz), 1.476 (18H, m), 1.438 (9H, s), 0.880 (9H, d, *J* = 1.2 Hz), 0.054 (6H, d, *J* = 3.6 Hz). ^{31}P-NMR ($CDCl_3$): δ = -6.13, -6.48. ^{13}C-NMR ($CDCl_3$): δ = 168.49, 168.47, 155.37, 83.72, 83.65, 82.43, 82.40, 79.74, 79.70, 71.19, 77.14, 71.66, 71.63, 71.61, 71.57, 71.53, 67.48, 67.42, 67.38, 62.31, 62.25, 62.15, 62.09, 59.04, 58.98, 54.50, 54.48, 54.40, 29.79, 29.76, 28.32, 27.97, 27.94, 25.79, 18.22, -5.48, -5.50, -5.54. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_{26}H_{54}NNaO_{10}PSi^+$: 622.3147. Found: 622.3152.

Synthesis of 48



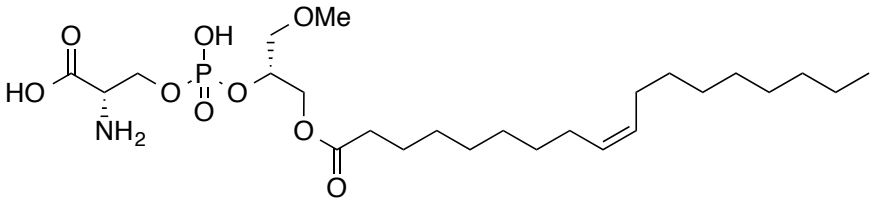
The alocohol **48** was synthesized from **47** in 73% yield in a similar manner to **33**. **48**: White solid. ^{1}H-NMR ($CDCl_3$): δ = 5.578 (1H, dd, *J* = 7.8, 23.4 Hz), 4.465 (1H, m), 4.373-4.178 (3H, m), 3.769 (2H, m), 3.527 (2H, m), 3.365 (3/2H, s), 3.360 (3/2H, s), 1.499-1.436 (27H, m). ^{31}P-NMR ($CDCl_3$): δ = -5.26, -5.41. ^{13}C-NMR ($CDCl_3$): δ = 168.48, 168.39, 155.28, 84.56, 84.49, 84.25, 84.18, 82.78, 82.74, 79.97, 79.91, 78.48, 78.42, 78.37, 72.10, 72.05, 71.99, 67.73, 67.71, 67.68, 67.65, 67.64, 67.58, 63.35, 63.32, 63.23, 63.21, 59.15, 54.46, 54.38, 29.74, 29.70, 28.29, 27.92. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_{20}H_{40}NNaO_{10}P^+$: 508.2282. Found: 508.2282.

Synthesis of 49



49 was synthesized from **48** in 83 % yield in a similar manner to **34**. **49**: Colorless oil. ^{1}H-NMR ($CDCl_3$): δ = 5.605 (1H, m), 5.340 (2H, m), 4.669-4.540 (1H, m), 4.377-4.164 (5H, m), 3.556 (2H, dt, *J* = 1.6, 5.2 Hz), 3.377 (3H, m), 2.328 (2H, m), 2.005 (4H, m), 1.626 (2H, m), 1.484 (18H, m), 1.446 (9H, s), 1.282 (20H, m), 0.877 (3H, t, *J* = 6.8 Hz). ^{31}P-NMR ($CDCl_3$): δ = -6.21, -6.55. ^{13}C-NMR ($CDCl_3$): δ = 173.30, 173.28, 168.41, 168.39, 155.34, 155.31, 130.00, 129.98, 129.73, 129.70, 82.55, 82.53, 79.84, 79.81, 74.64, 74.57, 74.50, 74.45, 71.67, 71.63, 71.60, 71.56, 67.68, 67.62, 67.58, 67.56, 67.51, 63.40, 63.34, 63.26, 63.20, 59.20, 59.16, 54.47, 54.38, 34.07, 34.01, 31.89, 29.80, 29.77, 29.75, 29.73, 29.70, 29.51, 29.31, 29.29, 29.20, 29.17, 29.11, 28.33, 27.97, 27.95, 27.20, 27.17, 24.81, 22.66, 14.09. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_{38}H_{72}NNaO_{11}P^+$: 772.4735. Found: 772.4729.

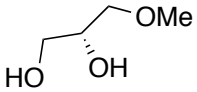
Synthesis of 4*R*



4*R* was synthesized from **49** in 83 % yield by global deprotection in TFA in a similar manner to **2*R***. **4*R*** : the TFA salt (white solid). ^{1}H-NMR ($CDCl_3$): δ = 5.359 (2H, m), 4.698 (2H, m), 4.544 (1H, m), 4.446 (1H, m), 4.358 (1H, m), 4.281 (1H, m), 3.762 (2H, s), 3.520 (3H, s), 2.419 (2H, d, *J* = 7.6 Hz), 2.016 (4H, m), 1.595 (2H, m), 1.284 (20H, m), 0.872 (3H, t, *J* = 6.8 Hz). ^{31}P-NMR ($CDCl_3$): δ = -1.96. HRMS (ESI-TOF, $[M-H]^-$): Calcd for $C_{25}H_{47}NO_9P^-$: 536.2994. Found: 536.3009. Mp. 192.5°C-196.0°C, colorless solid. Anal. Calcd. For $C_{25}H_{48}NO_9P \cdot 7.5CF_3COOH$: C, 34.49; H, 4.02; N, 1.01. Found: C, 34.39; H, 4.35; N, 1.14.

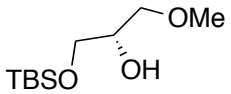
Synthesis of 4*S* (*O*-(hydroxy(((*S*)-1-methoxy-3-(oleoyloxy)propan-2-yl)oxy)-phosphoryl)-*L*-serine (1-methoxy-3-oleoyl LysoPS (S)) (Figure 26)

Synthesis of 50



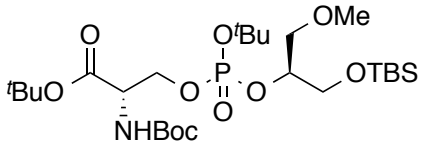
To a solution of (S)-(+)-2,2-dimethyl-1,3-dioxane-4-methanol (599.2 mg, 4.534 mmol) in anhydrous THF (10 mL), NaH (243.4 mg, 6.083 mmol) was added at 0ºC under an argon atmosphere. After 1 h, methyl iodide (966.7 mg, 6.811 mmol) was added and the mixture was stirred for 15 h at room temperature. MeOH (10 mL) was added and the solution was acidified with concentrated sulfuric acid. After 9 h, concentrated sulfuric acid was added and stirring was continued for 3 hours. MeOH was evaporated and the residue was chromatographed ($CHCl_3$: MeOH = 9:1). The residue was dissolved in MeOH (10 mL) and $AgNO_3$ (1.1666 g, 6.868 mmol) was added. The mixture was stirred for 7.5 hours, and then filtered through Celite®. The filtrate was evaporated and the residue was chromatographed ($CHCl_3$: MeOH = 9:1) to yield **50** (247.2 mg, 2.329 mmol, colorless oil, 51%). ^{1}H-NMR ($CDCl_3$): δ = 3.842 (1H, m), 3.654 (1H, dd, *J* = 3.6, 11.6 Hz), 3.562 (1H, dd, *J* = 6.0, 11.6 Hz), 3.425 (2H, m), 3.358 (3H, s).^{13}C-NMR ($CDCl_3$): δ = 74.14, 70.60, 63.95, 59.16. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_9H_{14}NaO_5^+$: 129.0522. Found: 129.0561.

Synthesis of 51



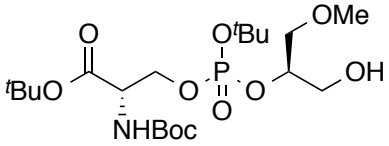
The diol **50** (137.2 mg, 1.293 mmol), iodine (322.1 mg, 2.538 mmol) and N-methylimidazole (294 µL, 320.5 mg, 3.903 mnol) were dissolved in anhydrous CH_2Cl_2 (5 mL) and the mixture was cooled to 0ºC. TBSCl (225.2 mg, 1.494 mmol) was added at 0ºC. The mixture was stirred for 8.5 h under an argon atmosphere at room temperature. To the solution, saturated $Na_2S_2O_3$ aq. (20 mL) was added and the whole was extracted three times with CH_2Cl_2 (total 80 mL). The combined organic layer was washed with brine (20 mL). The combined aqueous layer was extracted with CH_2Cl_2 (20 mL). The whole organic solution was dried over Na_2SO_4 and filtered. The filtrate was evaporated and the residue was chromatographed (n-hexane:AcOEt = 4:1) to yield **51** (184.5 mg, 0.837 mmol, 65%, colorless oil). ^{1}H-NMR ($CDCl_3$): δ = 3.798 (1H, m), 3.621 (2H, m), 3.410 (2H, m), 3.363 (3H, s), 0.885 (9H, s), 0.057 (6H, S). ^{13}C-NMR ($CDCl_3$): δ = 73.45, 70.54, 64.01, 59.13, 25.82, 18.25, -5.48. HRMS (ESI-TOF, $[M+Na]^+$):Calcd. for $C_{10}H_{24}NaO_3Si^+$: 243.1387. Found: 243.1391.

Synthesis of 52



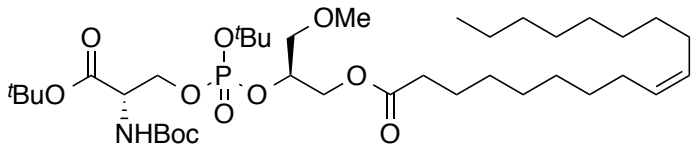
52 was synthesized from **51** and **28** in 54 % yield in a similar manner to **32**. **52**: Colorless oil. ^{1}H-NMR ($CDCl_3$): δ = 5.723 (1H, m), 4.428-4.275 (3H, m), 4.198 (1H, m), 3.705 (2H, m), 3.587-3.479 (2H, m), 3.320 (3H, m), 1.454-1.403 (27H, m), 0.846 (9H, s), 0.023 (3H, s), 0.019 (3H, s). ^{13}C-NMR ($CDCl_3$): δ = 168.40, 168.36, 155.29, 155.27, 83.76, 83.69, 83.60, 83.53, 82.32, 82.26, 79.59, 79.56, 77.17, 77.10, 71.78, 71.73, 71.47, 71.42, 67.39, 67.33, 67.18, 67.12, 62.30, 62.25, 62.02, 61.97, 58.90, 58.87, 54.45, 54.38, 54.30, 29.68, 29.65, 29.64, 29.61, 28.20, 27.83, 27.81, 25.68, 18.12, 18.10, -5.61, -5.64. ^{31}P-NMR ($CDCl_3$): δ = -6.078, -6.231. HRMS (ESI-TOF, $[M+Na]^+$):Calcd. for $C_{26}H_{54}NNaO_{10}PSi^+$: 622.3147. Found: 622.3144.

Synthesis of 53



The alocohol **53** was synthesized from **52** in 43% yield in a similar manner to **33**. **53:** White solid. ^{1}H-NMR ($CDCl_3$): δ = 5.630 (1H, m), 4.466 (1H, m), 4.348 (2H, m), 4.260 (2H, m), 3.778 (2H, m), 3.543 (2H, m), 3.380 (3/2H, s), 3.363 (3/2H, s), 1.480 (27H, m). ^{13}C-NMR ($CDCl_3$): δ = 168.52, 168.47, 168.43, 155.48, 155.33, 84.63, 84.55, 84.42, 84.35, 82.77, 82.67, 82.64, 79.95, 79.89, 78.49, 78.42, 78.35, 72.12, 72.06, 72.02, 71.96, 67.74, 67.69, 67.65, 67.58, 63.08, 63.05, 63.00, 62.96, 62.93, 59.13, 59.09, 54.45, 54.37, 29.73, 29.68, 28.27, 27.90, 27.89, 27.87. ^{31}P-NMR ($CDCl_3$): δ = -5.300, -5.605. HRMS (ESI-TOF, $[M+Na]^+$):Calcd. for $C_{20}H_{40}NNaO_{10}P^+$: 508.2282. Found: 508.2268. Mp: 62.5-63.5ºC, white powder.

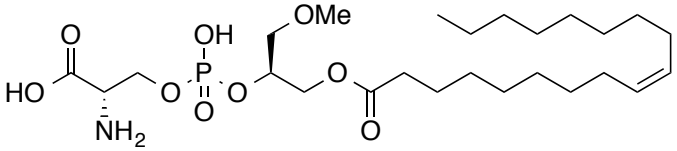
Synthesis of 54



54 was synthesized from **53** and oleoyl chloride in 20 % yield in a similar manner to **34**. **54:** Colorless oil. ^{1}H-NMR ($CDCl_3$): δ = 5.615 (1H, m), 5.338 (2H, m), 4.607 (1H, m), 4.371-4.160 (5H, m), 3.552 (2H, m), 3.384 (3/2H, s), 3.366 (3/2H, s), 2.332 (2H, m), 1.619 (2H, m), 1.471

(27H, m), 1.341-1.252 (20H, m), 0.876 (3H, t, J = 6.8 Hz). ^{13}C-NMR ($CDCl_3$): δ = 173.26, 168.41, 168.37, 155.31, 129.97, 129.96, 129.70, 129.68, 84.22, 84.15, 84.06, 83.98, 82.55, 82.49, 79.79, 74.67, 74.61, 74.48, 74.43, 71.75, 71.71, 71.59, 71.55, 67.58, 67.52, 67.46, 67.41, 63.34, 63.28, 63.22, 63.17, 59.17, 59.14, 54.51, 54.44, 54.36, 34.04, 33.98, 31.86, 29.76, 29.72, 29.67, 29.61, 29.57, 29.48, 29.44, 29.28, 29.27, 29.17, 29.15, 29.08, 28.30, 27.97, 27.94, 27.91, 27.18, 27.14, 24.79, 22.63, 14.06. ^{31}P-NMR ($CDCl_3$): δ =-6.14, -6.36. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_{38}H_{72}NNaO_{11}P^+$: 772.4735. Found: 772.4737.

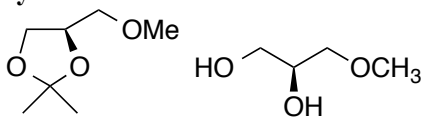
Synthesis of 4*S*



4*S* was synthesized from **54** in 90 % yield by global deprotection in TFA in a similar manner to **2*R***. **4*S*** : the TFA salt (brown solid). ^{1}H-NMR ($CDCl_3$/TFA-d): δ = 5.368 (1H, m), 4.698 (3H, m), 4.556 (1H, brs), 4.446 (1H, dd, J = 3.2, 12 Hz), 4.266 (1H, d, J = 10.4 Hz), 3.764 (2H, brs), 3.521 (3H, s), 2.416 (2H, t, J = 6.8 Hz), 2.007 (2H, m), 1.662 (4H, m), 1.305-1.270 (20H, m), 0.875 (3H, t, J = 6.6 Hz). ^{31}P-NMR ($CDCl_3$/TFA-d): δ = -2.39. HRMS (ESI-TOF, $[M-H]^-$): Calcd for $C_{25}H_{47}NO_9P^-$: 536.2994. Found: 536.3003. Mp: 149.0°C-150.5°C, colorless cubes. Anal. Calcd. For $C_{25}H_{48}NO_9P \cdot 1.1CF_3COOH$: C, 49.27; H, 7.46; N, 2.11. Found: C, 49.18; H, 7.39; N, 2.28.

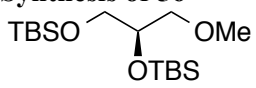
Synthesis of 5*R* (*O*-(hydroxy((*R*)-3-methoxy-2-(oleoyloxy)propoxy)phosphoryl)-*L*-serine (1-methoxy-2-oleoyl-LysoPS)) (Figure 27)

Synthesis of 55



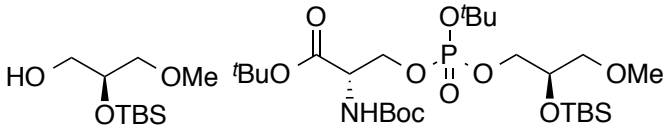
(R)-(-)-2,2-Dimethyl-1,3-dioxolane-4-methanol (104.4 mg, 0.790 mmol) was dissolved in anhydrous THF (3.0 ml) and cooled to 0°C. NaH (68.4 mg, 1.708 mmol) was added, and the reaction mixture was stirred for 0.5 h at 0°C under an argon atmosphere. MeI was added, and stirring was continued at room temperature for 3.5 h. Amberlyst (115.7 mg) and MeOH (4 ml) were then added, and stirring was continued for 23 h. Amberlyst (127.4mg) was added, and the mixture was stirred for another 2 h and filtered through Celite. The filtrate was evaporated. The residue was chromatographed ($CHCl_3$/MeOH = 10:1 to 5:1) to yield **55** (67.5 mg, 0.636 mmol, 81%). ^{1}H-NMR ($CDCl_3$): δ = 3.867 (1H, m), 3.710 (1H, dd, J = 3.8, 7.4 Hz), 3.631 (1H, dd, J = 5.6, 7.2 Hz), 3.483 (2H, m), 3.392 (3H, brs). ^{13}C-NMR ($CDCl_3$): δ = 74.29, 70.45, 64.06, 59.24. HRMS (ESI-TOF, $[M+Na]^+$): Calcd for $C_4H_{10}NaO_3^+$: 129.0522. Found: 129.0541.

Synthesis of 56



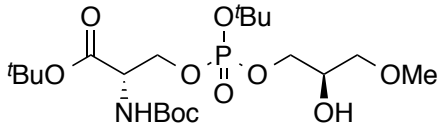
56 was synthesized from the diol **55** in 82 % yield in a similar manner to **31**. **56**: Colorless oil. ^{1}H-NMR ($CDCl_3$): δ = 3.803 (1H, m), 3.584 (1H, dd, J = 5.8, 10.2 Hz), 3.525 (1H, dd, J = 5.6, 10.0 Hz), 3.432 (1H, dd, J = 4.4, 6.0 Hz), 3.344 (3H,s), 3.330 (1H, dd, J = 5.4, 9.8 Hz), 0.892 (9H, s), 0.887 (9H, s), 0.077 (6H, d, J = 0.8 Hz), 0.049 (6H, s). ^{13}C-NMR ($CDCl_3$): δ = 74.67, 72.62, 64.99, 59.16, 25.94, 25.87, 18.34, 18.22, -4.62, -4.73, -5.37, -5.43. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_{16}H_{38}NaO_3Si_2^+$: 357.2252. Found: 357.2244.

Synthesis of 57 and 58



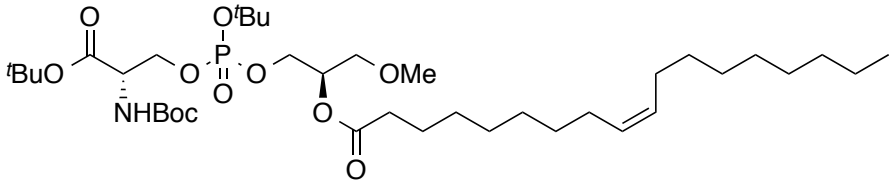
56 (416.3 mg, 1.244 mmol) was dissolved in MeOH (20 mL) and cooled to 0°C. Camphorsulfonic acid (14.6 mg, 0.063 mmol) was added, and the mixture was stirred for 1.5 h at 0°C. Triethylamine (4 mL) was then added, and the reaction mixture was evaporated. The residue was chromatographed (n-hexane:AcOEt = 1:0, 20:1 to 4:1) to yield crude **57** (107.5 mg), which was used without further purification. **28** (178.9 mg, 0.385 mmol) was dissolved in CH_2Cl_2 and co-evaporated with toluene. The alcohol **57** (105.3 mg) was dissolved in CH_2Cl_2 and co-evaporated with toluene. Under an argon atmosphere, the mixture was dissolved in CH_2Cl_2 (1.5 mL), and a solution of 1*H*-tetrazole (35.3 mg, 0.504 mmol) in THF (1.5 mL) was added at room temperature. After a few minutes, a white solid precipitated. The reaction mixture was stirred for 2.75 h at room temperature. TBHP in decane (5.0-6.0 M) (0.154 ml, 0.770 mmol) was added, and stirring was continued for 12 h at room temperature. The solution was diluted with water (5 mL) and extracted three times with CH_2Cl_2 (5 mL). The combined organic layer was washed with brine. The combined aqueous layer was extracted once with CH_2Cl_2 (5 mL). The whole organic layer was dried over Na_2SO_4, and filtered. The filtrate was evaporated and the residue was chromatographed (n-hexane:acetone = 4:1) to yield **58** (162.2 mg, 0.270 mmol, 70 %, white solid). ^{1}H-NMR ($CDCl_3$): δ = 5.517 (1H, m), 4.339 (2H, m), 4.245-4.158 (1H, m), 4.018-3.839 (3H, m), 3.411-3.330 (5H, m), 1.474 (18H, m), 1.436 (9H, s), 0.875 (9H, s), 0.078 (6H, m). ^{31}P-NMR ($CDCl_3$): δ = -5.63, -5.82. ^{13}C-NMR ($CDCl_3$): δ = 168.37, 168.34, 155.37, 155.31, 155.27, 83.61, 83.58, 83.51, 82.56, 82.54, 79.82, 73.79, 73.76, 70.35, 70.26, 68.46, 68.39, 68.33, 67.40, 67.34, 59.18, 59.16, 54.50, 54.45, 54.42, 54.36, 29.80, 29.76, 29.73, 28.31, 27.94, 25.74, 18.10, -4.77, -4.82. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_{26}H_{54}NNaO_{10}PSi^+$: 622.3147. Found: 622.3130. Mp. 58.0°C-62.0℃, white solid. Anal. Calcd. for $C_{26}H_{54}NO_{10}PSi$: C, 52.07; H, 9.07; N, 2.34. Found: C, 51.81; H, 8.90; N, 2.25.

Synthesis of 59



The alocohol **59** was synthesized from **58** in 16 % yield in a similar manner to **33**. **59:** White solid. ^{1}H-NMR ($CDCl_3$): δ = 5.530 (1H, dd, *J* = 7.8, 22.2 Hz), 4.380-4.318 (2H, m), 4.289-4.220 (2H, m), 4.105-3.949 (3H, m), 3.444 (2H, m), 3.378 (3/2H, s), 3.374 (3/2H, s), 1.485 (18H, m), 1.445 (9H, s). ^{31}P-NMR ($CDCl_3$): δ = -4.86, -4.92. ^{13}C-NMR ($CDCl_3$): δ = 168.39, 168.36, 155.23, 84.23, 84.15, 84.07, 82.83, 82.76, 79.98, 72.77, 69.33, 69.27, 68.94, 68.92, 68.89, 68.84, 67.93, 67.62, 67.57, 59.21, 54.46, 54.39, 29.77, 29.76, 29.73, 29.71, 28.29, 27.93. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_{20}H_{40}NNaO_{10}P^+$: 508.2282. Found: 508.2300.

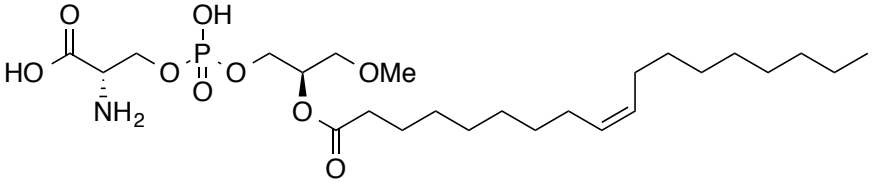
Synthesis of 60



60 was synthesized from **59** and oleoyl chloride in 73 % yield in a similar manner to **34**. **60**: Colorless oil. ^{1}H-NMR ($CDCl_3$): δ = 5.542 (1H, t, *J* = 8.2 Hz), 5.338 (2H, m), 5.136 (1H, m), 4.335 (2H, m), 4.249-4.054 (3H, m), 3.529 (2H, t, *J* = 5.2 Hz), 3.355 (3H, d, *J* = 2.8 Hz), 2.333 (2H, m), 2.002 (4H, m), 1.617 (2H, quintet, *J* = 7.3Hz), 1.468 (27H, m), 1.342-1.262 (20H, m), 0.875 (3H, t, *J* = 7.0 Hz). ^{31}P-NMR ($CDCl_3$): δ = -5.81, -5.96. ^{13}C-NMR ($CDCl_3$): δ = 172.99, 168.32, 155.29, 129.99, 129.72, 84.03, 83.96, 83.90, 82.66, 82.64, 79.92, 70.45, 70.42, 70.37, 70.34, 70.29, 70.27, 67.55, 67.52, 67.50, 65.37, 65.32, 65.26, 59.24, 54.46, 54.41, 54.37, 34.21,

31.89, 29.77, 29.75, 29.73, 29.70, 29.64, 29.61, 29.57, 29.50, 29.46, 29.30, 29.29, 29.18, 29.12, 29.06, 28.31, 27.95, 27.21, 27.17, 24.85, 24.83, 22.66, 14.09. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_{38}H_{72}NNaO_{11}P^+$: 772.4735. Found: 772.4702.

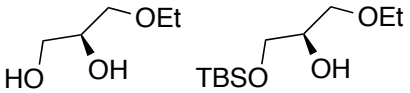
Synthesis of 5*R*



5*R* was synthesized from **60** in 68 % yield by global deprotection in TFA in a similar manner to **2*R***. **5*R*** : the TFA salt (white solid). ^{1}H-NMR ($CDCl_3$): δ = 5.371 (2H, m), 4.614 (3H, m), 4.173 (2H, d, *J* = 15.2 Hz), 3.774 (3H, brs), 3.533 (3H, s), 2.417 (2H, t, *J* = 7.4 Hz), 2.006 (4H, m), 1.595 (2H, m), 1.351-1.274 (20H, m), 0.875 (3H, t, *J* = 6.4 Hz). ^{31}P-NMR ($CDCl_3$): δ = -1.60. HRMS (ESI-TOF, $[M-H]^-$): Calcd for $C_{25}H_{47}NO_9P^-$: 536.2994. Found: 536.2995.

Synthesis of LysoPS Ether Derivatives 6 (*O*-((((*R*)-1-ethoxy-3-(oleoyloxy)propan-2-yl)oxy)(hydroxy)phosphoryl)-*L*-serine), 7 (*O*-(hydroxy(((*R*)-1-(oleoyloxy)-3-(prop-2-yn-1-yloxy)propan-2-yl)oxy)phosphoryl)-*L*-serine), 8 (*O*-((((*R*)-1-(benzyloxy)-3-(oleoyloxy)-propan-2-yl)oxy)(hydroxy)phosphoryl)-*L*-serine), 9 (*O*-((((*R*)-1-(benzyloxy)-3-(oleoyloxy)-propan-2-yl)oxy)(hydroxy)phosphoryl)-*L*-serine) (Figure 28)

Figure 28 (A)
Synthesis of 61 and 62



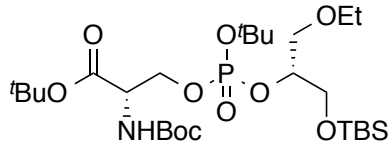
To a solution of (R)-(+)-2,2-dimethyl-1,3-dioxane-4-methanol (219.2 mg, 1.659 mmol) and TBAI (184.2 mg, 0.499 mmol) in anhydrous DMF (5 mL), NaH (136.8 mg, 3.421 mmol) was added at 0°C under an argon atmosphere. After 0.5 h, ethyl bromide (248 μL, 362.1 mg, 3.323 mmol) was added and the mixture was stirred for 0.5 h at 0°C and 7 h at room temperature. H_2O (30 mL) was added, and the mixture was extracted three times with diethyl ether (15 mL). The combined organic layer was washed with brine (15 mL). The combined aqueous layer was extracted with diethyl ether (15 mL). The whole organic solution was dried over Na_2SO_4 and filtered. The filtrate was evaporated and the residue was dissolved in anhydrous MeOH (5 mL). To this solution, Amberlyst® 15 (217.5 mg) was added, and the mixture was stirred for 14.5 h at room temperature, then filtered through Celite®. The filtrate was evaporated and chromatographed (AcOEt to $CHCl_3$:MeOH = 9:1) to yield crude **61**. This was used without further purification. Crude **61** and imidazole (100.7 mg, 1.479 mmol) were dissolved in anhydrous DMF (3 mL). At 0°C, TBSCl (184.8 mg, 1.226 mmol) was added to the solution. The mixture was stirred for 0.5 h at 0°C and for 12.5 h at room temperature under an argon atmosphere. H_2O (20 mL) was added at 0°C and the mixture was extracted three times with diethyl ether (20 mL). The combined organic layer was washed with brine (20 mL), dried over Na_2SO_4, and filtered. The combined aqueous layer was extracted three times with diethyl ether (total 60 mL). The whole organic solution was washed with brine (20 mL), dried over Na_2SO_4 and filtered. The filtrate was evaporated and chromatographed (n-hexane: AcOEt = 1:0 to 9:1). The residue was chromatographed (n-hexane:AcOEt = 20:1) to yield **62** (137.0 mg, 0.584 mmol, 46% (3 steps), yellow oil).

61: ^{1}H-NMR ($CDCl_3$): δ = 3.843 (1H, m), 3.668 (1H, dd, *J* = 3.6 Hz, 11.6 Hz), 3.578 (1H, dd, *J* = 5.8 Hz, 11.4 Hz), 3.543-3.428 (4H, m), 1.182 (3H, t, *J* = 7.0 Hz). ^{13}C-NMR ($CDCl_3$): δ = 71.99, 70.63, 66.90, 64.03, 14.97. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_5H_{12}NaO_3^+$: 143.0679. Found: 143.0706.

62: ^{1}H-NMR ($CDCl_3$): δ = 3.805 (1H, m), 3.637 (2H, m), 3.545-3.413 (4H, m), 2.497 (1H, d, *J* = 4.8 Hz), 1.199 (3H, t, *J* = 7.0 Hz), 0.893 (9H, s), 0.066 (6H, s). ^{13}C-NMR ($CDCl_3$): δ = 71.24,

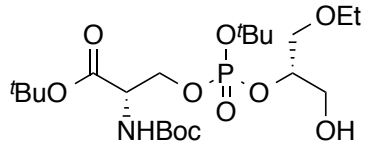
70.66, 66.78, 64.06, 25.85, 18.28, 15.09, -5.45. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_{11}H_{26}NaO_3Si^+$: 257.1543. Found: 257.1535.

Synthesis of 63



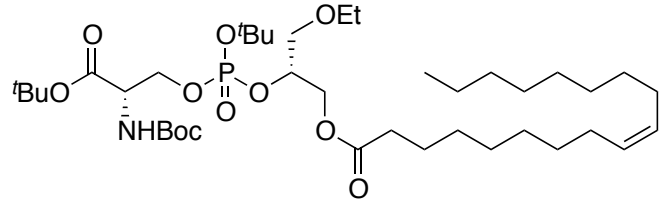
63 was synthesized from **62** and **28** in 71 % yield in a similar manner to **32**. **63**: Thick oil. ^{1}H-NMR ($CDCl_3$): δ = 5.593 (1H, m), 4.452-4.303 (3H, m), 4.213 (1H, m), 3.732 (2H, m), 3.651-3.429 (4H, m), 1.468-1.421 (27H, m), 1.168 (3H, dt, *J* = 0.8, 7.0 Hz), 0.863 (9H, d, *J* = 0.8 Hz), 0.042 (3H, s), 0.033 (3H, s). ^{13}C-NMR ($CDCl_3$): δ = 168.47, 168.40, 155.35, 155.30, 83.72, 83.68, 83.64, 83.61, 82.42, 82.39, 79.73, 79.67, 77.39, 69.29, 69.24, 69.20, 67.41, 67.34, 67.27, 66.66, 62.34, 62.29, 62.19, 62.14, 54.49, 54.46, 54.41, 54.39, 29.76, 29.72, 28.28, 27.92, 27.91, 25.77, 18.20, 15.00, -5.51, -5.53, -5.56. ^{31}P-NMR ($CDCl_3$): δ = -6.144, -6.397. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_{27}H_{56}NNaO_{10}PSi^+$: 636.3303. Found: 636.3286.

Synthesis of 64



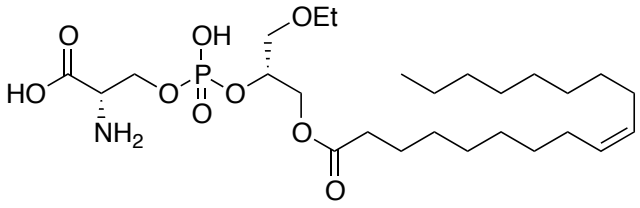
The alocohol **64** was synthesized from **63** in 39 % yield in a similar manner to **33**. **64:** White sticky solid. ^{1}H-NMR ($CDCl_3$): δ = 5.563 (1H, m), 4.449 (1H, m), 4.382-4.221 (3H, m), 3.832-3.715 (2H, m), 3.618-3.447 (4H, m), 1.463 (27H, m), 1.171 (3H, t, *J* = 7.0 Hz). ^{13}C-NMR ($CDCl_3$): δ = 168.47, 168.40, 155.28, 84.50, 84.43, 84.21, 84.13, 82.77, 82.72, 79.97, 79.91, 78.58, 78.51, 78.45, 69.98, 69.92, 69.86, 67.68, 67.62, 67.55, 66.91, 66.87, 63.46, 63.43, 63.35, 63.33, 54.47, 54.40, 29.74, 29.71, 28.28, 27.92, 15.00. ^{31}P-NMR ($CDCl_3$): δ = -5.338, -5.397. HRMS (ESI-TOF, $[M+Na]^+$):Calcd. for $C_{21}H_{42}NNaO_{10}P^+$: 522.2439. Found: 522.2392. Mp: 83.5-85.0°C, white paste.

Synthesis of 65 (Figure 28 (D))



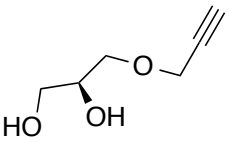
65 was synthesized from **64** and oleoyl chloride in 77 % yield in a similar manner to **34**. **65**: Colorless oil. ^{1}H-NMR ($CDCl_3$): δ = 5.582 (1H, m), 5.321 (2H, m), 4.592 (1H, m), 4.322 (3H, m), 4.208 (2H, m), 3.531 (4H, m), 2.316 (2H, m), 1.991 (4H, m), 1.597 (2H, m), 1.463 (27H, m), 1.330-1.238 (20H, m), 1.174 (3H, dt, *J* = 1.2 Hz, 7.0 Hz), 0.863 (3H, t, *J* = 6.8 Hz). ^{13}C-NMR ($CDCl_3$): δ = 173.32, 173.28, 168.39, 168.34, 155.32, 155.27, 129.97, 129.95, 129.70, 129.68, 84.12, 84.05, 83.97, 82.52, 79.83, 79.79, 74.76, 74.69, 74.67, 74.61, 69.39, 69.36, 69.33, 67.62, 67.57, 67.49, 67.44, 66.90, 63.51, 63.45, 63.42, 63.37, 54.46, 54.37, 34.06, 34.00, 31.86, 29.77, 29.75, 29.73, 29.71, 29.67, 29.48, 29.27, 29.17, 29.15, 29.09, 28.29, 27.93, 27.18, 27.14, 24.80, 22.63, 14.98, 14.06. ^{31}P-NMR ($CDCl_3$): δ = -6.219, -6.477. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_{39}H_{74}NNaO_{11}P^+$: 786.4892. Found: 786.4858.

Synthesis of 6



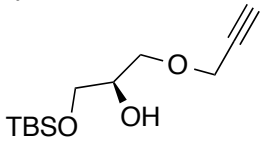
6 was synthesized from **65** in 90 % yield by global deprotection in TFA in a similar manner to **2*R***. **6** : the TFA salt (white solid). ^{1}H-NMR ($CDCl_3$/TFA-d): δ = 5.372 (2H, m), 4.692 (3H, m), 4.567 (1H, brs), 4.436 (1H, dd, *J* = 3.4, 11.8 Hz), 4.281 (1H, dd, *J* = 3.6, 12.4 Hz), 3.769 (4H, m), 2.425 (2H, m), 2.007 (2H, m), 1.649 (4H, m), 1.306-1.246 (23H, m), 0.876 (3H, t, *J* = 6.6 Hz). ^{31}P-NMR ($CDCl_3$/TFA-d): δ = -2.27. HRMS (ESI-TOF, $[M-H]^-$): Calcd. for $C_{26}H_{50}NO_9P^-$: 550.3150. Found: 550.3174. Mp. 130.2°C-131.0 °C, colorless solid. Anal. Calcd. For $C_{26}H_{50}NO_9P \cdot 0.7CF_3COOH$: C, 52.12; H, 8.09; N, 2.22. Found: C, 51.96; H, 7.97; N, 2.17.

Figure 28 (B)
Synthesis of 66



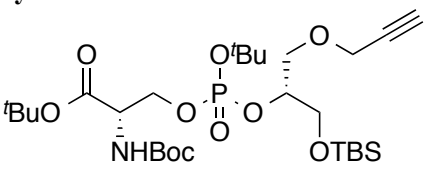
To a solution of (R)-(+)-2,2-dimethyl-1,3-dioxane-4-methanol (520.2 mg, 3.936 mmol) in anhydrous DMF (6 mL) was added NaH (395.5 mg, 9.888 mmol) at 0°C under an argon atmosphere. After 0.5 h, propargyl bromide (594 μL, 938.5 mg, 7.889 mmol) was added and the reaction mixture was stirred for 3.5 h at room temperature. H_2O (40 mL) was added, and the mixture was extracted three times with Et_2O (30 mL). The combined organic layer was washed with brine (30 mL). The combined aqueous layer was extracted with Et_2O (30 mL). The whole organic solution was dried over Na_2SO_4 and filtered. The filtrate was evaporated and the residue was dissolved in anhydrous MeOH (6 mL). Amberlyst® 15 (741.2 mg) was added, and the mixture was stirred for 18 h at room temperature under an argon atmosphere, then filtered through Celite®. The filtrate was evaporated, and the residue was chromatographed (n-hexane: AcOEt = 2:1 to $CHCl_3$: MeOH = 9:1) to yield **66** (477.5 mg, 3.669 mmol, 93%, brown solution). ^{1}H-NMR ($CDCl_3$): δ = 4.176 (2H, d, *J* = 2.0 Hz), 3.885 (1H, m), 3.683 (1H, m), 3.578 (3H, m), 3.372 (1H, d, *J* = 4.0 Hz), 3.026 (1H, t, *J* = 5.6 Hz), 2.463 (1H, t, *J* = 2.2 Hz). ^{13}C-NMR ($CDCl_3$): δ = 79.24, 74.94, 71.27, 70.61, 63.76, 58.58. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_6H_{10}NaO_3^+$: 153.0522. Found: 153.0522.

Synthesis of 67



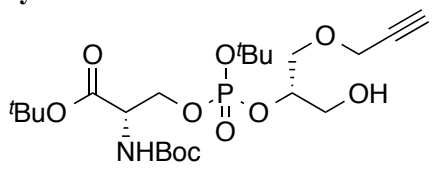
67 was synthesized from the diol **66** in 85 % yield in a similar manner to **31**. **67**: Colorless oil. ^{1}H-NMR ($CDCl_3$): δ = 4.189 (2H, d, *J* = 2.4 Hz), 3.843 (1H, m), 3.689-3.526 (4H, m), 2.436 (1H, t, *J* = 2.2 Hz). ^{13}C-NMR ($CDCl_3$): δ = 79.46, 74.63, 70.67, 70.53, 63.88, 58.57, 25.84, 18.26, -5.44. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_{12}H_{24}NaO_3Si^+$: 267.1387. Found: 267.1398.

Synthesis of 68



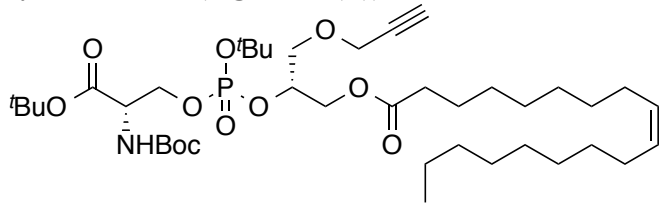
68 was synthesized from **67** and **28** in 80 % yield in a similar manner to **32**. **68**: Colorless oil. ^{1}H-NMR ($CDCl_3$): δ = 5.549 (1H, m), 4.466-4.308 (3H, m), 4.241-4.121 (3H, m), 3.796-3.692 (3H, m), 3.652 (1H, ddd, *J* = 2.8, 5.5 Hz, 10.5 Hz), 2.399 (1H, m), 1.459 (9H, dd, *J* = 0.2, 2.2 Hz), 1.442 (9H, s), 1.416 (9H, s), 0.855 (9H, d, *J* = 1.2 Hz), 0.037 (3H, s), 0.029 (3H, s). ^{13}C-NMR ($CDCl_3$): δ = 168.42, 168.37, 155.26, 83.81, 83.77, 83.74, 83.70, 82.40, 79.75, 79.71, 79.31, 79.29, 76.97, 74.76, 74.69, 68.71, 68.66, 68.64, 68.59, 67.46, 67.39, 67.33, 62.18, 62.13, 62.02, 61.97, 58.32, 58.26, 54.41, 54.34, 54.32, 31.50, 29.76, 29.72, 29.69, 28.26, 27.90, 27.89, 25.74, 22.56, 18.15, 14.03, -5.53, -5.55, -5.58. ^{31}P-NMR ($CDCl_3$): δ = -6.18, -6.47. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_{28}H_{54}NNaO_{10}PSi^+$: 646.3147. Found: 646.3158.

Synthesis of 69



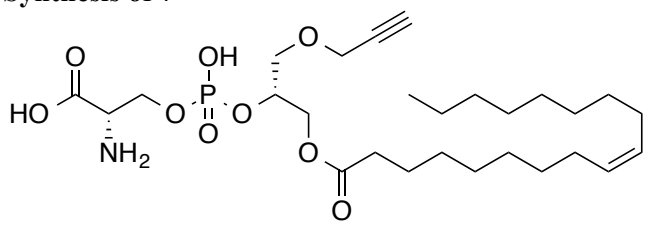
The alocohol **69** was synthesized from **68** in 67 % yield in a similar manner to **33**. **69:** Thick oil. ^{1}H-NMR ($CDCl_3$): δ = 5.554 (1H, m), 4.432 (1H, m), 4.331-4.165 (3H, m), 4.128 (2H, d, *J* = 2.4 Hz), 3.765-3.626 (4H, m), 3.314 (1H, brs), 2.411 (1H, quartet, *J* = 2.4 Hz), 1.443-1.382 (27H, m). ^{13}C-NMR ($CDCl_3$): δ = 168.36, 168.29, 155.17, 84.43, 84.36, 84.18, 84.10, 82.62, 82.56, 79.84, 79.78, 79.03, 79.01, 78.28, 78.24, 78.22, 78.18, 74.98, 74.90, 68.92, 68.88, 68.85, 68.82, 67.58, 67.52, 67.48, 67.43, 62.72, 62.69, 62.66, 58.31, 58.29, 54.33, 54.25, 29.64, 29.62, 29.60, 29.59, 28.17, 27.80. ^{31}P-NMR ($CDCl_3$): δ = -5.65, -5.70. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_{22}H_{40}NNaO_{10}P^+$: 532.2282. Found: 532.2282.

Synthesis of 70 (Figure 28 (D))



70 was synthesized from **69** and oleoyl chloride in 84 % yield in a similar manner to **34**. **70:** Colorless oil. ^{1}H-NMR ($CDCl_3$): δ = 5.595 (1H, t, *J* = 9.2 Hz), 5.341 (2H, m), 4.640 (1H, m), 4.402-4.158 (7H, m), 3.718 (2H, m), 2.466 (1H, quartet, *J* = 2.2 Hz), 2.337 (2H, quartet, *J* = 8.1 Hz), 2.009 (4H, quartet, *J* = 6.3 Hz), 1.629 (2H, m), 1.504-1.452 (27H, m), 1.344-1.269 (20H, m), 0.880 (3H, t, *J* = 6.8 Hz). ^{13}C-NMR ($CDCl_3$): δ = 173.27, 173.24, 168.42, 168.38, 155.32, 155.30, 130.00, 129.99, 129.73, 129.72, 84.29, 84.21, 84.14, 82.58, 82.57, 79.89, 79.87, 79.05, 79.03, 75.20, 75.12, 74.51, 74.45, 74.43, 74.37, 68.70, 68.65, 68.64, 68.60, 67.73, 67.67, 67.59, 67.54, 63.34, 63.28, 63.22, 63.17, 58.52, 58.46, 54.47, 54.39, 34.07, 34.01, 31.90, 29.82, 29.78, 29.77, 29.71, 29.52, 29.32, 29.21, 29.19, 29.13, 28.34, 27.97, 27.22, 27.18, 24.82, 22.68, 14.12. ^{31}P-NMR ($CDCl_3$): δ = -6.28, -6.55. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_{40}H_{72}NNaO_{11}P^+$: 796.4735. Found: 796.4737.

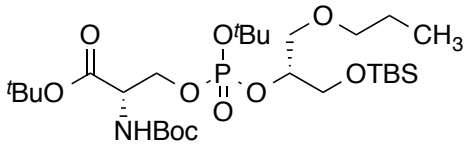
Synthesis of 7



7 was synthesized from **70** in 88 % yield by global deprotection in TFA in a similar manner to **2*R***. **7** : the TFA salt (white solid). ^{1}H-NMR ($CDCl_3$/TFA-d): δ = 5.354 (2H, m), 4.636 (3H, brs), 4.433 (2H, m), 4.228 (3H, s), 3.761 (2H, m), 2.516 (1H, s), 2.389 (2H, t, *J* = 7.6 Hz), 1.986 (4H, m),

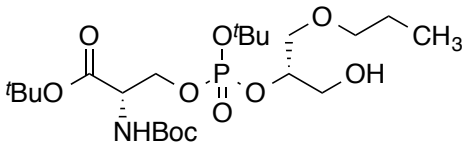
1.636 (2H, m), 1.346-1.266 (20H, m), 0.873 (3H, t, J = 6.8 Hz). ^{31}P-NMR ($CDCl_3$/TFA-d): δ = -2.87. HRMS (ESI-TOF, $[M-H]^-$): Calcd for $C_{27}H_{47}NO_9P^-$: 560.2994. Found: 560.3029. Mp: 142.5°C-146.0°C, colorless cubes. Anal. Calcd. for $C_{27}H_{48}NO_9P \cdot 0.8CF_3COOH$: C, 52.62; H, 7.35; N, 2.15. Found: C, 52.98; H, 7.57; N, 2.13.

Synthesis of 71



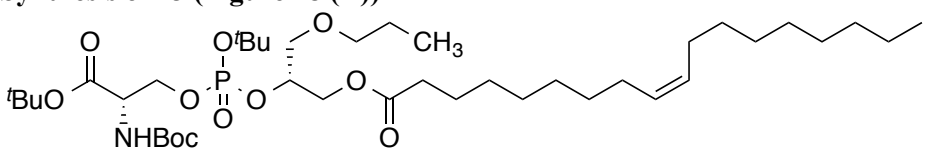
68 (403.3 mg, 0.647 mmol) and $Pd(OH)_2$ (36.0 mg) were dissolved in AcOEt (12 mL). Under a hydrogen atmosphere, the reaction mixture was stirred at room temperature for 11 h, then filtered through Celite®. The filtrate was evaporated, and the residue was chromatographed (n-hexane:AcOEt = 3:1) to yield **71** (347.3 mg, 0.553 mmol, 85%, thick oil). ^{1}H-NMR ($CDCl_3$): δ = 5.570 (1H, m), 4.444-4.306 (3H, m), 4.201 (1H, m), 3.740 (2H, m), 3.592 (2H, m), 3.378 (2H, m), 1.564 (2H, m), 1.462-1.415 (27H, m), 0.895-0.856 (12H, m), 0.036 (3H, s), 0.026 (3H, s). ^{13}C-NMR ($CDCl_3$): δ = 168.44, 168.37, 155.32, 155.27, 83.65, 83.63, 83.58, 83.56, 82.39, 82.36, 79.70, 79.64, 77.37, 73.08, 73.05, 69.39, 69.35, 69.31, 67.39, 67.33, 67.27, 62.31, 62.26, 62.17, 62.12, 54.46, 54.44, 54.39, 54.36, 29.75, 29.71, 28.27, 27.91, 25.77, 22.71, 18.19, 10.45, -5.53, -5.56. ^{31}P-NMR ($CDCl_3$): δ = -6.10, -6.35. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_{28}H_{58}NNaO_{10}PSi^+$: 650.3460. Found: 650.3443.

Synthesis of 72



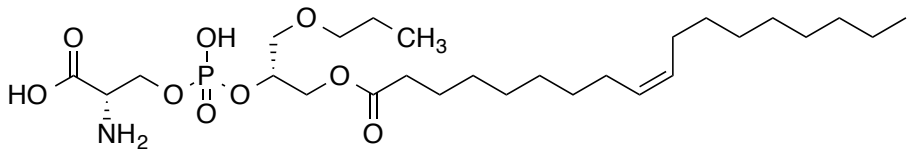
The alocohol **72** was synthesized from **71** in 72 % yield in a similar manner to **33**. **72:** Thick oil. ^{1}H-NMR ($CDCl_3$): δ = 5.586 (1H, m), 4.477 (1H, m), 4.407-4.243 (3H, m), 3.806 (2H, m), 3.579 (2H, m), 3.429 (2H, m), 3.062 (1H, brs), 1.584 (2H, m), 1.515-1.453 (27H, m), 0.906 (3H, m). ^{13}C-NMR ($CDCl_3$): δ = 168.45, 168.37, 155.25, 84.47, 84.39, 84.17, 84.09, 82.75, 82.70, 79.94, 79.88, 78.55, 78.48, 78.41, 73.25, 73.21, 70.17, 70.11, 70.04, 67.67, 67.60, 67.54, 63.47, 63.44, 63.37, 63.34, 54.42, 54.34, 29.71, 29.68, 28.25, 27.89, 22.72, 22.68, 10.41. ^{31}P-NMR ($CDCl_3$): δ = -5.30, -5.36. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_{22}H_{44}NNaO_{10}P^+$: 536.2595. Found: 536.2603. Mp: 81.0-83.0°C, colorless paste.

Synthesis of 73 (Figure 28 (D))



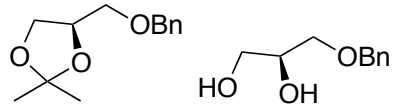
73 was synthesized from **72** and oleoyl chloride in 37 % yield in a similar manner to **34**. **73:** Colorless oil. ^{1}H-NMR ($CDCl_3$): δ = 5.589 (1H, m), 5.343 (2H, m), 4.609 (1H, m), 4.339 (3H, m), 4.226 (2H, m), 3.591 (2H, m), 3.418 (2H, m), 2.334 (2H, m), 2.008 (2H, m), 1.597 (4H, m), 1.490-1.450 (27H, m), 1.345-1.267 (22H, m), 0.893 (3H, m). ^{13}C-NMR ($CDCl_3$): δ = 173.40, 173.36, 168.43, 168.39, 155.36, 155.32, 130.02, 130.00, 129.75, 129.73, 84.17, 84.10, 84.03, 82.59, 79.90, 79.85, 74.78, 74.72, 74.69, 74.64, 73.32, 69.57, 69.55, 69.53, 67.67, 67.62, 67.55, 67.50, 63.56, 63.49, 63.43, 54.48, 54.40, 34.11, 34.05, 31.92, 29.82, 29.78, 29.76, 29.73, 29.53, 29.33, 29.23, 29.21, 29.14, 28.34, 27.97, 27.23, 27.20, 24.85, 22.75, 22.74, 22.69, 14.12, 10.48. ^{31}P-NMR ($CDCl_3$): δ = -6.17, -6.44. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_{40}H_{76}NNaO_{11}P^+$: 800.5048. Found: 800.5044.

Synthesis of 8



8 was synthesized from **73** in a quantitative yield by global deprotection in TFA in a similar manner to **2*R***. **8** : the TFA salt (white solid). ^{1}H-NMR ($CDCl_3$/TFA-d): δ = 5.363 (2H, m), 4.635 (3H, brs), 4.514 (1H, brs), 4.437 (1H, m), 4.259 (1H, d, *J* = 10 Hz), 3.769 (2H, m), 3.613 (2H, m), 2.403 (2H, t, *J* = 7.4 Hz), 1.994 (4H, m), 1.630 (4H, m), 1.285 (20H, m), 0.888 (6H, m). ^{31}P-NMR ($CDCl_3$/TFA-d): δ = -2.83. HRMS (ESI-TOF, $[M-H]^-$): Calcd for $C_{27}H_{51}NO_9P^-$: 564.3307. Found: 564.3343. Mp: 139.0°C-142.0°C, colorless cubes. Anal. Calcd. for $C_{27}H_{52}NO_9P \cdot CF_3COOH$: C, 51.25; H, 7.86; N, 2.06. Found: C, 51.00; H, 7.94; N, 2.00.

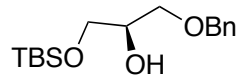
Figure 28 (C)
Synthesis of 74



To a solution of (R)-(+)-2,2-dimethyl-1,3-dioxane-4-methanol (203.3 mg, 1.538 mmol) in anhydrous THF (3 mL), NaH (124.7 mg, 3.118 mmol) was added at 0°C under an argon atmosphere. After 0.5 h, benzyl bromide (330.1 mg, 1.930 mmol) in anhydrous THF (3 mL) was added. The reaction mixture was stirred for 14.5 h at room temperature, then H_2O (15 mL) was added at 0°C, and the mixture was extracted three times with AcOEt (10 mL). The combined organic layer was washed with brine (10 mL). The combined aqueous layer was extracted with AcOEt (10 mL). The whole organic solution was dried over Na_2SO_4 and filtered. The filtrate was evaporated and the residue was chromatographed (n-hexane:AcOEt = 15:1) to yield the acetal form of **74** (304.3 mg, 1.369 mmol, 89%, colorless oil). ^{1}H-NMR ($CDCl_3$): δ = 7.373-7.266 (5H, m), 4.579 (2H, dd, *J* = 12.2 Hz), 4.309 (1H, m), 4.060 (1H, dd, *J* = 6.4, 8.4 Hz), 3.747 (1H, dd, *J* = 6.2, 8.2 Hz), 3.560 (1H, dd, *J* = 5.6, 9.6 Hz), 3.477 (1H, dd, *J* = 5.6, 9.6 Hz), 1.426 (3H, s), 1.369 (3H, s) ^{13}C-NMR ($CDCl_3$): δ = 137.92, 128.37, 127.70, 127.68, 109.36, 74.70, 73.47, 71.04, 66.83, 26.73, 25.35. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_{13}H_{18}NaO_3^+$: 245.1148. Found: 245.1148.

The acetal form of **74** (303.0 mg, 1.363 mmol) was dissolved in anhydrous MeOH (6 mL). Amberlyst® 15 (205.3 mg) was added, and the mixture was stirred for 15.5 h at room temperature, then filtered through Celite® and the filtrate was evaporated. The residue was chromatographed (n-hexane: AcOEt = 1:1, 0:1 to $CHCl_3$:MeOH = 9:1) to yield **74** (231.0 mg, 1.268 mmol, 93%, colorless oil). ^{1}H-NMR ($CDCl_3$): δ = 7.376-7.276 (5H, m), 4.545 (2H, s), 3.886 (1H, m), 3.689 (1H, m), 3.634-3.505 (3H, m), 2.907 (1H, brs), 2.459 (1H, brs). ^{13}C-NMR ($CDCl_3$): δ = 137.63, 128.48, 127.89, 127.77, 73.55, 71.74, 70.62, 64.02. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_{10}H_{14}NaO_3^+$: 205.0835. Found: 205.0835.

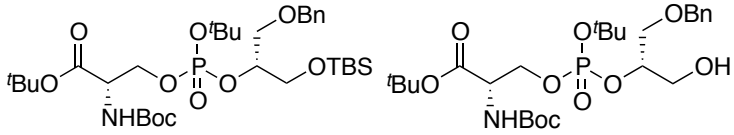
Synthesis of 75



A mixture of **74** (180.1 mg, 0.988 mmol), iodine (259.6 mg, 2.046 mmol) TBSCl (166.2 mg, 1.103 mmol) and N-methylimidazole (246 μL, 268.1 mg, 3.266 mnol) in anhydrous CH_2Cl_2 (4 mL) was stirred for 3 h under an argon atmosphere at room temperature. Saturated $Na_2S_2O_3$ aq. (20 mL) was added and the resulting mixture was extracted three times with CH_2Cl_2 (20 mL). The combined organic layer was washed with brine (20 mL). The combined aqueous layer was extracted with CH_2Cl_2 (20 mL). The whole organic solution was dried over Na_2SO_4 and filtered. The filtrate was evaporated and the residue was chromatographed (n-hexane:AcOEt = 9:1 to 4:1) to yield **75** (219.0 mg, 0.739 mmol, 75%, colorless oil). ^{1}H-NMR ($CDCl_3$): δ = 7.375-7.268 (5H, m), 4.557 (2H, s), 3.857 (1H, m), 3.662 (2H, m), 3.527 (2H, m), 2.516 (1H, d, *J* = 4.8 Hz), 0.895 (9H, s), 0.069 (6H, S). ^{13}C-NMR ($CDCl_3$): δ = 138.03, 128.39, 127.71, 127.69, 73.42, 70.93,

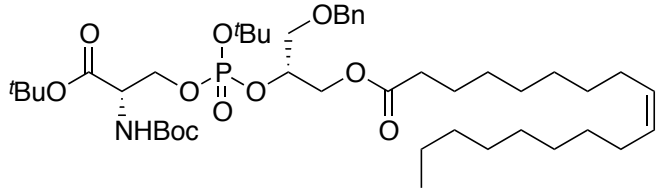
70.67, 63.98, 25.84, 18.26, -5.44. HRMS (ESI-TOF, $[M+Na]^+$):Calcd. for $C_{16}H_{28}NaO_3Si^+$: 319.1700. Found: 319.1692.

Synthesis of 76 and 77



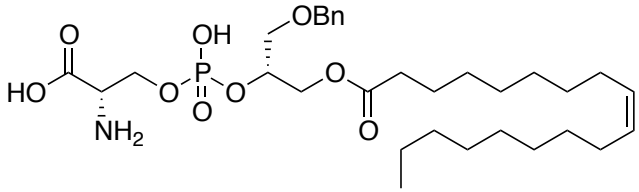
Alcohol **75** (135.0 mg, 0.455 mmol) was dissolved in CH_2Cl_2 and co-evaporated with toluene. **28** (204.9 mg, 0.441 mmol) was added, and the mixture was dissolved in CH_2Cl_2 and co-evaporated with toluene. Under an argon atmosphere, the residue was dissolved in anhydrous CH_2Cl_2 (5 mL), and 1*H*-tetrazole (71.5 mg, 1.021 mmol) in anhydrous THF (5 mL) was added. After a few minutes, a white solid precipitated. The reaction mixture was stirred for 11.5 h at room temperature, then diluted with saturated $NaHCO_3$ aq. (15 ml) and extracted three times with CH_2Cl_2 (total 50 ml). The combined organic layer was washed with brine (20 mL). The combined aqueous layer was extracted with CH_2Cl_2 (20 mL). The whole organic solution was dried over Na_2SO_4 and filtered. The filtrate was evaporated and the residue was chromatographed (n-hexane:AcOEt:Et_3N = 35:4:1). The residue was dissolved in anhydrous CH_2Cl_2 (5 mL). TBHP in decane (5.0-6.0 M) (185 μL, 0.925 mmol) was added, and the mixture was stirred for 4.5h at room temperature under an argon atmosphere. The solution was diluted with water (15 mL) and extracted three times with CH_2Cl_2 (15 mL). The combined organic layer was washed with brine (20 mL). The combined aqueous layer was extracted with CH_2Cl_2 (20 mL). The whole organic solution was dried over Na_2SO_4 and filtered. The filtrate was evaporated and the residue was chromatographed (n-hexane:AcOEt = 4:1) to yield crude **76**, which was used without further purification. **76** was dissolved in anhydrous MeOH (5.5 mL), and Amberlyst® 15 (709.5 mg) was added at 0°C. The mixture was stirred for 14 h at room temperature, then filtered through Celite®. The filtrate was evaporated, and the residue was chromatographed (n-hexane:AcOEt = 4:1, 2:1 to n-hexane:acetone = 2:1) to yield **77** (52.6 mg, 0.094 mmol, 21% (2 steps), thick oil). ^{1}H-NMR ($CDCl_3$): δ = 7.356-7.249 (5H, m), 5.562 (1H, m), 4.504 (3H, m), 4.346 (2H, m), 4.265 (1H, m), 3.792 (2H, m), 3.615 (2H, m), 1.460 (27H, m). ^{13}C-NMR ($CDCl_3$): δ = 168.47, 168.40, 155.27, 137.69, 137.60, 128.40, 128.37, 127.78, 127.73, 127.66, 127.63, 84.58, 84.50, 84.26, 84.18, 82.80, 82.75, 80.01, 79.95, 78.70, 78.64, 78.57, 78.51, 73.33, 69.46, 69.40, 69.34, 67.70, 67.64, 67.58, 63.37, 63.34, 63.28, 63.25, 54.46, 54.38, 29.73, 29.69, 28.28, 27.91. ^{31}P-NMR ($CDCl_3$): δ = -5.302. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_{26}H_{44}NNaO_{10}P^+$: 584.2595. Found: 584.2578. Mp: 77.0-78.5°C, white paste.

Synthesis of 78 (Figure 28 (D))



78 was synthesized from **77** and oleoyl chloride in 49 % yield in a similar manner to **34**. **78:** Colorless oil. ^{1}H-NMR ($CDCl_3$): δ = 7.355-7.251 (5H, m), 5.573 (1H, m), 5.334 (2H, m), 4.632 (1H, m), 4.536 (2H, m), 4.331 (3H, m), 4.221 (2H, m), 3.627 (2H, m), 2.280 (2H, m), 1.996 (4H, m), 1.587 (2H, t, *J* = 6.6 Hz), 1.447 (29H, m), 1.351-1.258 (18H, m), 0.868 (3H, t, *J* = 6.8 Hz). ^{13}C-NMR ($CDCl_3$): δ = 173.28, 173.25, 168.38, 168.33, 155.30, 155.27, 137.61, 137.55, 129.98, 129.95, 129.71, 129.68, 128.40, 128.37, 127.79, 127.74, 127.68, 84.19, 84.14, 84.12, 84.06, 82.55, 79.87, 79.84, 74.63, 74.58, 74.55, 74.50, 73.33, 73.28, 68.87, 68.86, 68.83, 68.80, 68.75, 67.54, 63.35, 63.29, 63.22, 54.44, 54.36, 34.02, 33.96, 31.86, 29.76, 29.74, 29.73, 29.70, 29.68, 29.48, 29.28, 29.27, 29.18, 29.15, 29.10, 29.08, 28.30, 28.29, 27.94, 27.92, 27.18 27.15, 27.14, 24.76, 22.64, 14.07. ^{31}P-NMR ($CDCl_3$): δ = -6.159, -6.404. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_{44}H_{76}NNaO_{11}P^+$: 848.5048. Found: 848.5033.

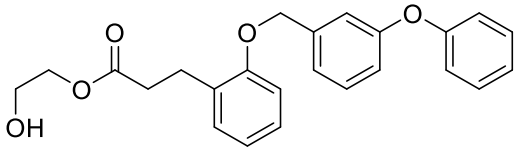
Synthesis of 9



9 was synthesized from **78** in 98 % yield by global deprotection in TFA in a similar manner to **2*R***. **9** : the TFA salt (brown solid). ^{1}H-NMR ($CDCl_3$): δ = 7.390-7.320 (5H, m), 5.380 (2H, m), 4.665 (5H, m), 4.409 (1H, brd, *J* = 9.2 Hz), 4.265 (2H, m), 3.803 (2H, m), 2.368 (2H, t, *J* = 7.6 Hz), 2.025 (2H, m), 1.674 (2H, m), 1.570 (2H, brt, *J* = 6.2 Hz), 1.348-1.281 (20H, m), 0.879 (3H, t, *J* = 6.6 Hz). ^{31}P-NMR ($CDCl_3$): δ = -2.462. HRMS (ESI-TOF, $[M-H]^-$): Calcd for $C_{31}H_{51}NO_9P^-$: 612.3307. Found: 612.3278. Mp. 118.5-122.0°C, colorless cubes. Anal. Calcd. For $C_{31}H_{52}NO_9P \cdot 0.9CF_3COOH$: C, 54.99; H, 7.44; N, 1.96. Found: C, 55.14; H, 7.51; N, 1.86.

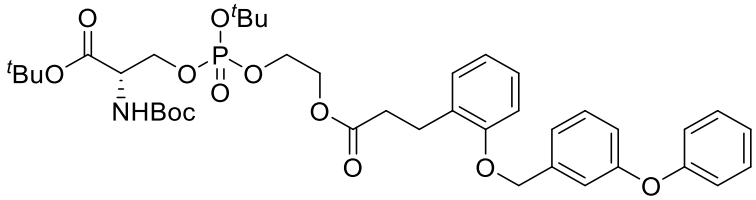
Synthesis of 10 (*O*-(hydroxy(2-((3-(2-((3-phenoxybenzyl)oxy)phenyl) propanoyl)oxy)ethoxy)phosphoryl)-*L*-serine (deoxy-LysoPS-C2-C3-ph-o-O-Bn-m-O-ph)) (Figure 29)

Synthesis of 79



Ethylene glycol (18.40 mg, 0.296 mmol) and carboxylic acid **A (Figure 9, C3-ph-o-O-Bn-m-O-ph)** (103.0 mg, 0.296 mmol) were dissolved in anhydrous CH_2Cl_2 (1.5 mL). DMAP (3.60 mg, 0.030 mmol), and EDCI・HCl (68.0 mg, 0.355 mmol) were added, and the mixture was stirred at room temperature for 24 h. The reaction was quenched with water (8 mL) and the mixture was extracted three times with CH_2Cl_2 (8 mL). The combined organic layer was washed with brine (8 mL). The combined aqueous layer was extracted with CH_2Cl_2 (8 mL). The whole organic solution was dried over Na_2SO_4 and filtered. The filtrate was evaporated and the residue was chromatographed (n-hexane:AcOEt = 2:1) to yield **79** (87.80 mg, 0.224 mmol, 76%, colorless oil). ^{1}H-NMR ($CDCl_3$): δ = 7.378-7.333 (2H, m), 7.216-7.109 (5H, m), 7.064-7.028 (2H, m), 6.971 (1H, dd, *J* = 8.4, 2.0 Hz), 6.963-6.877 (2H, m), 5.077 (2H, s), 4.174-4.151 (2H, m), 3.745-3.722 (2H, m), 3.010 (2H, t, *J* = 7.6 Hz), 2.684 (2H, t, *J* = 7.6 Hz), 2.027 (1H, brs). ^{13}C-NMR ($CDCl_3$): δ = 173.61, 157.69, 156.92, 156.41, 139.30, 130.21, 129.97, 129.85, 129.01, 127.75, 123.54, 121.63, 120.92, 119.18, 117.99, 117.19, 111.71, 69.39, 66.04, 61.17, 34.13, 26.31. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_{24}H_{24}NaO_5^+$: 415.1516. Found: 415.1526.

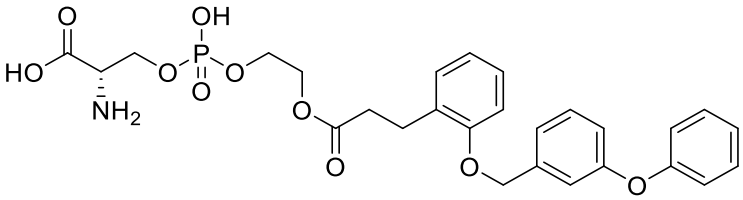
Synthesis of 80



The alcohol **79** (82.7 mg, 0.211 mmol) was dissolved in CH_2Cl_2 and co-evaporated with toluene. To the residue, **28** (79.3 mg, 0.176 mmol), CH_2Cl_2 and toluene were added, and the solvent was evaporated under vacuum. Under an argon atmosphere, the mixture was dissolved in anhydrous CH_2Cl_2 (1.8 mL), and 1*H*-tetrazole (36.9 mg, 0.527 mmol) in anhydrous THF (1.8 mL) was added at room temperature. After a few minutes, a white solid precipitated. The reaction mixture was stirred for 4 h at room temperature, then TBHP in decane (5.0-6.0 M) (70.2 μL, 0.351 mmol) was added, and stirring was continued for 1.5 h at room temperature under an argon atmosphere. The

reaction mixture was diluted with saturated $NaHCO_3$ aq (6 ml) and the solution was extracted three times with CH_2Cl_2 (6 ml). The combined organic layer was washed with brine (6 mL). The combined aqueous layer was extracted with CH_2Cl_2 (6 mL). The whole organic solution was dried over Na_2SO_4 and filtered. The filtrate was evaporated and the residue was chromatographed (n-hexane: acetone = 4:1) to yield **80** (90.4 mg, 0.117 mmol, 67 %, colorless oil). ^{1}H-NMR ($CDCl_3$): δ = 7.349-7.310 (3H, m), 7.175-7.085 (4H, m), 7.055 (1H, m), 7.021-7.000 (2H, m), 6.951-6.926 (1H, m), 6.900-6.838 (2H, m), 5.505-5.486 (1H, m), 5.056 (2H, s), 4.364-4.246 (2H, m), 4.236-4.195 (3H, m), 4.173-4.075 (2H, m), 2.973 (2H, t, *J* = 7.6 Hz), 2.667-2.620 (2H, m), 1.472-1.428 (27H, m). ^{13}C-NMR ($CDCl_3$): δ = 172.90, 172.88, 168.32, 157.65, 156.89, 156.36, 155.24, 139.31, 130.16, 129.95, 129.81, 128.98, 128.96, 127.64, 123.48, 121.54, 120.87, 119.14, 117.93, 117.09, 111.62, 84.06, 83.99, 82.71, 82.68, 79.94, 69.32, 67.52, 67.46, 65.01, 64.95, 62.88, 62.87, 62.80, 62.79, 54.48, 54.39, 33.90, 29.78, 29.76, 29.74, 29.72, 28.31, 27.95, 27.94, 25.99. ^{31}P-NMR ($CDCl_3$): δ = -5.68, -5.63. HRMS (ESI-TOF, $[M-H]^-$): Calcd for $C_{40}H_{54}NNaO_{12}P^+$: 794.3276. Found: 794.3261.

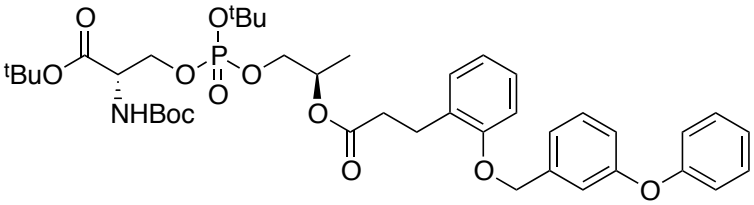
Synthesis of 10



80 (90.3 mg, 0.117 mmol) was dissolved in TFA (2 mL) at 0 ^{0}C and the solution was stirred at 0 ^{0}C for 10 min, then at room temperature for 1.5 h. The solvent was removed under vacuum and the residue was chromatographed ($CHCl_3$:MeOH:AcOH = 6:1:2 to 4:1:4) to yield **10** as the AcOH salt (58.5 mg, 0.105 mmol, 89%, white solid). The AcOH salt was dissolved in TFA and evaporated to yield **10** as the TFA salt. ^{1}H-NMR ($CDCl_3$): δ = 7.354-7.315 (3H, m), 7.205-7.074 (5H, m), 7.016-6.995 (2H, m), 6.955 (1H, dd, *J* = 8.0 Hz, 2.0 Hz), 6.914-6.878 (2H, m), 5.072 (2H, s), 4.529 (2H, m), 4.398 (1H, m), 4.276 (2H, m), 4.162-4.144 (2H, m), 2.962 (2H, t, *J* = 7.6 Hz), 2.723 (2H, t, *J* = 7.6 Hz). ^{31}P-NMR ($CDCl_3$): δ = -1.23. HRMS (ESI-TOF, $[M-H]^-$): Calcd for $C_{27}H_{29}NO_{10}P^-$: 558.1535. Found: 558.1531. Anal. Calcd. for $C_{25}H_{42}NO_9P \cdot 0.8CF_3COOH$: C, 52.78; H, 4.73; N, 2.15. Found: C, 52.60; H, 4.88; N, 2.15.

Synthesis of LPS1 selective agonists (1-deoxy-2-acyl o-OBn-m-OPh and 1-deoxy-3-acyl o-OBn-m-OPh) 11 (1-deoxy-2-acyl o-OBn-m-OPh: *O*-(hydroxy((*R*)-2-((3-(2-((3-phenoxybenzyl)oxy)phenyl)propanoyl)oxy)propoxy)phosphoryl)-*L*-serine) (Figure 30)

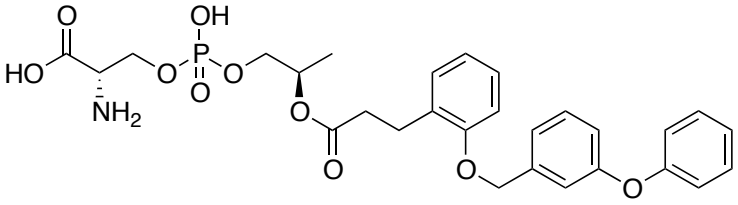
Synthesis of 82



81 (91.2 mg, 0.200 mmol), acid **A** (104.0 mg, 0.299 mmol) and 4-dimethylaminopyridine (DMAP) (3.6 mg, 0.0296 mmol) were dissolved in CH_2Cl_2 (2 mL). EDCI (68.8 mg, 0.359 mmol) was added, and the mixture was stirred for 20 h at room temperature. Then MeOH (1 mL) and EDCI (30.6 mg, 0.178 mmol) were added, and stirring was continued for 5 hours at room temperature. The reaction mixture was diluted with H_2O (10 mL) and the whole was extracted three times with CH_2Cl_2 (10 mL x 3). The combined organic layer was washed with brine and dried over Na_2SO_4, and the solvent was evaporated. The residue was column-chromatographed (n-hexane: ethyl acetate = 4:1) to yield **82** (147.4 mg, 0.188 mmol, 94%, colorless oil). ^{1}H-NMR ($CDCl_3$): δ = 7.338 (3H, m), 7.177-7.092 (4H, m), 7.059 (1H, m), 7.029-7.005 (2H, m), 6.043 (1H, dd, *J* = 8.12, 1.72 Hz), 6.905-6.842 (2H, m), 5.502 (1H, m), 5.069 (3H, m), 4.346 (2H, m),

4.209 (1H, m), 3.956 (2H, m), 2.969 (2H, t, *J* = 7.52 Hz), 2.284 (2H, dt, *J* = 7.70, 2.80 Hz), 1.462 (18H, s), 1.435 (9H, m), 1.209 (3/2H, d, *J* = 6.40 Hz), 1.197 (3/2H, d, *J* = 6.40 Hz). ^{31}P-NMR ($CDCl_3$): δ = -5.681, -5.864. ^{13}C-NMR ($CDCl_3$): δ = 172.45, 168.32, 157.64, 156.89, 156.37, 155.26, 139.33, 130.16, 129.91, 129.78, 129.04, 127.57, 123.46, 121.50, 119.13, 117.88, 117.07, 111.61, 82.66, 79.93, 69.33, 68.33, 68.76, 68.68, 68.61, 67.51, 54.39, 34.17, 29.77, 29.73, 28.31, 27.94, 26.04, 16.23. HRMS (ESI, $[M+Na]^+$): Calcd for $C_{41}H_{56}NNaO_{12}P^+$: 808.3432. Found: 808.3436.

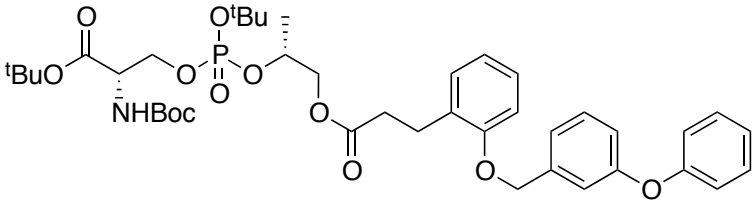
Synthesis of 11



82 (139.7 mg, 0.178 mmol) was dissolved in trifluoroacetic acid (TFA) (1 mL) and the mixture was stirred for 1 h at room temperature. The reaction mixture was evaporated and the residue was column-chromatographed ($CHCl_3$: methanol:acetic acid = 8:1:1) to yield **11** as the acetic acid salt (65.0 mg, 0.0583 mmol, 64%, white powder). The acetic acid salt was dissolved in TFA and the acid was evaporated to yield **11** as the TFA salt (white powder). ^{1}H-NMR ($CDCl_3$/TFA-d = 4:1): δ= 7.298 (3H, m), 7.111 (4H, m), 7.036 (1H, m), 6.985 (2H, m), 6.937 (1H, m), 6.835 (2H, m), 5.027 (3H, m), 4.359 (2H, m), 4.247 (1H, m), 3.846 (2H, m), 2.923 (2H, t, *J* = 6.78 Hz), 2.643 (2H, m), 1.078 (3H, m). ^{31}P-NMR ($CDCl_3$/TFA-d = 4:1): δ = -1.704. HRMS (ESI, $[M-H]^-$): Calcd for $C_{28}H_{31}NO_{10}P^-$: 572.1691. Found: 572.1682. Mp: 171.0-172.0 ºC. Anal. Calcd. For $C_{28}H_{32}FNO_{10}P•CH_3COOH$: C, 56.87; H, 5.73; N, 2.21. Found: C, 56.99; H, 5.47; N, 2.35.

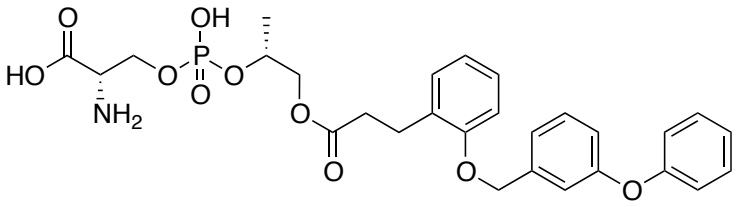
Synthesis of 12 (*O*-(hydroxy(((*R*)-1-((3-(2-((3-phenoxybenzyl)oxy)phenyl)-propanoyl)oxy)propan-2-yl)oxy)phosphoryl)-*L*-serine) (Figure 31)

Synthesis of 83



83 was synthesized from alcohol **33** and acid **A** in 94 % yield in a similar manner to **81**. **83**: Colorless oil. ^{1}H-NMR ($CDCl_3$): δ = 7.337 (3H, m), 7.184-7.094 (4H, m), 7.057 (1H, m), 7.021 (2H, m), 6.943 (1H, dd, *J* = 8.08, 2.36 Hz), 6.905-6.842 (2H, m), 5.502 (1H, m), 5.066 (2H, s), 4.596 (1H, m), 4.334 (2H, m), 4.204 (1H, m), 4.081 (2H, t, *J* = 5.76 Hz), 2.980 (2H, t, *J* = 7.62, 2.60 Hz), 2.652 (2H, m), 1.471-1.450 (18H, m), 1.438-1.426 (9H, m), 1.280 (3H, dd, *J* = 8.12, 6.48 Hz). ^{13}C-NMR ($CDCl_3$): δ = 172.81, 172.78, 168.35, 157.63, 157.61, 156.88, 156.34, 155.22, 139.30, 139.27, 130.11, 129.91, 129.77, 129.00, 128.98, 127.60, 127.56, 123.44, 123.43, 121.52, 121.50, 120.85, 117.89, 117.10, 117.08, 111.60, 83.75, 82.61, 82.57, 79.85, 72.61, 69.33, 67.39, 66.73, 66.70, 66.64, 54.46, 54.37, 33.90, 33.86, 29.80, 29.76, 29.70, 28.29, 27.92, 25.97, 25.93, 18.12, 18.09. ^{31}P-NMR ($CDCl_3$): δ = -6.077, -6.528. HRMS (ESI, $[M+Na]^+$): Calcd for $C_{41}H_{56}NNaO_{12}P^+$: 808.3432. Found: 808.3438.

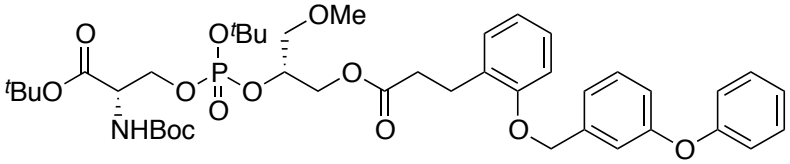
Synthesis of 12



12 was synthesized from **83** in 98 % yield by global deprotection in TFA in a similar manner to **11**. **12** : the TFA salt (white solid).^{1}H-NMR ($CDCl_3$/TFA-d = 4:1): δ = 7.360-7.321 (3H, m), 7.215-7.082 (5H, m), 7.014-6.899 (5H, m), 5.085 (2H, s), 4.604 (3H, m), 4.476 (1H, m), 4.172 (1H, m), 4.118 (1H, m), 2.972 (2H, t, *J* = 6.80 Hz), 2.738 (2H, t, *J* = 6.90 Hz), 1.297 (3H, m). ^{31}P-NMR ($CDCl_3$/TFA-d = 4:1): δ = -2.301. HRMS (ESI, $[M-H]^-$): Calcd for $C_{28}H_{31}NO_{10}P^-$: 572.1691. Found: 572.1709. Mp: 143.5-144.5 ºC. Anal. Calcd. For $C_{28}H_{32}NO_{10}P$•0.1CF_3COOH: C, 57.90; H, 5.53; N, 2.39. Found: C, 57.66; H, 5.63; N, 2.46.

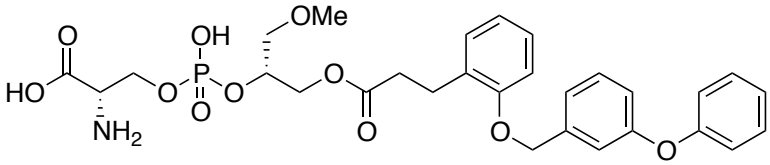
Synthesis of 13 (1-methoxy-3-acyl LysoPS C3-ph-o-O-Bn-m-O-ph: *O*-(hydroxy(((*R*)-1-methoxy-3-((3-(2-((3-phenoxybenzyl)oxy)phenyl)propanoyl)oxy)propan-2-yl)oxy)phosphoryl)-*L*-serine) (Figure 32)

Synthesis of 84



84 was synthesized from alcohol **48** and acid **A** in 75 % yield in a similar manner to **81**. **84**: Colorless oil. ^{1}H-NMR ($CDCl_3$): δ = 7.339 (3H, m), 7.137 (4H, m), 7.024 (3H, m), 6.900 (3H, m), 5.625 (1H, d, *J* = 8.4 Hz), 5.063 (2H, s), 4.578 (1H, m), 4.348 (2H, m), 4.291-4.158 (3H, m), 3.486 (2H, d, *J* = 4.8 Hz), 3.338 (3H, s), 2.975 (2H, dt, *J* = 2.4, 7.6 Hz), 2.646 (2H, quintet, *J* = 7.7 Hz), 1.472-1.429 (27H, m). ^{13}C-NMR ($CDCl_3$): δ = 172.70, 168.37, 157.62, 157.60, 156.85, 156.83, 156.32, 155.32, 155.30, 139.27, 139.24, 130.10, 129.92, 129.76, 128.93, 127.60, 127.57, 123.45, 123.44, 121.51, 121.48, 120.84, 119.11, 117.87, 117.06, 117.04, 111.57, 84.17, 84.10, 84.03, 82.52, 82.49, 79.81, 79.78, 74.55, 74.49, 74.44, 74.38, 71.58, 71.53, 71.50, 69.30, 67.66, 67.60, 67.52, 67.47, 63.39, 63.33, 63.17, 63.12, 59.13, 59.10, 54.43, 54.34, 33.86, 33.82, 29.76, 29.71, 29.67, 28.29, 27.92, 25.94, 25.91. ^{31}P-NMR ($CDCl_3$): δ = -6.22, -6.64. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_{42}H_{58}NNaO_{13}PSi^+$: 838.3538. Found: 838.3548.

Synthesis of 13

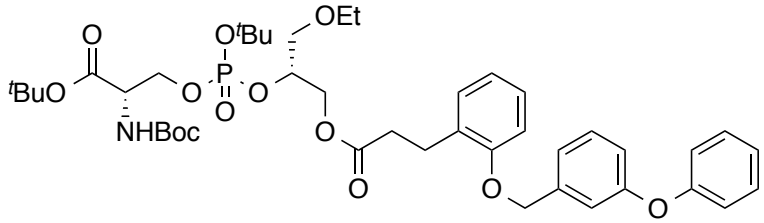


13 was synthesized from **84** in 90 % yield by global deprotection in TFA in a similar manner to **11**. **13** : the TFA salt (browwn solid). ^{1}H-NMR ($CDCl_3$): δ = 7.347 (3H, m), 7.228-7.087 (5H, m), 7.022-6.906 (5H, m), 5.102 (2H, s), 4.655 (3H, m), 4.530 (1H, brs), 4.338 (1H, dd, *J* = 4.4, 12.0 Hz), 4.224 (1H, dd, *J* = 4.6, 12.0 Hz), 3.634 (2H, m), 3.467 (3H, s), 2.978 (2H, t), 2.759 (2H, t, *J* = 7.4 Hz). ^{31}P-NMR ($CDCl_3$): δ = -2.27. HRMS (ESI-TOF, $[M-H]^-$): Calcd for $C_{29}H_{33}NO_{11}P^-$: 602.1797. Found: 602.1844. Mp: 51.5°C-53.5°C, colorless solid. Anal. Calcd. for $C_{29}H_{34}NO_{11}P$ • 1.5CF_3COOH: C, 49.62; H, 4.62; N, 1.81. Found: C, 49.40; H, 4.76; N, 1.81.

Synthesis of 14 (*O*-((((*R*)-1-ethoxy-3-((3-(2-((3-phenoxybenzyl)oxy)phenyl)-propanoyl)oxy)propan-2-yl)oxy)(hydroxy)phosphoryl)-*L*-serine: 1-ethoxy-3-acyl LysoPS C3-ph-o-O-Bn-m-O-ph)

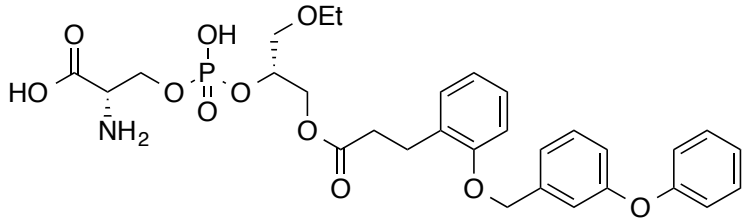
(Figure 33)

Synthesis of 85



A mixture of alcohol **64** (23.1 mg, 0.046 mmol), DMAP (3.9 mg, 0.032 mmol), EDCI・HCl (30.8 mg, 0.161 mmol) and acid **A** (24.1 mg, 0.069 mmol) in anhydrous CH_2Cl_2 (0.8 mL) was stirred under an argon atmosphere for 9 h at room temperature. Then MeOH (0.2 mL) was added and stirring was continued for 6.5 h. H_2O (5 mL) was added, and the mixture was extracted three times with CH_2Cl_2 (5 mL). The combined organic layer was washed with brine (5 mL). The combined aqueous layer was extracted three times with CH_2Cl_2 (5 mL). The whole organic solution was dried over Na_2SO_4 and filtered. The filtrate was evaporated and the residue was chromatographed (n-hexane:AcOEt = 3:1 to 2:1) to yield **85** (34.7 mg, 0.042 mmol, 91%, colorless oil). ^{1}H-NMR ($CDCl_3$): δ = 7.340 (3H, m), 7.190-7.094 (4H, m), 7.031 (3H, m), 6.944 (1H, dd, *J* = 2.4, 8.0 Hz), 6.877 (2H, m), 5.607 (1H, m), 5.064 (2H, s), 4.581 (1H, m), 4.377-4.170 (5H, m), 3.569-3.421 (4H, m), 2.978 (2H, dt, *J* = 2.8, 7.6 Hz), 2.649 (2H, m), 1.475-1.428 (27H, m), 1.169 (3H, dt, *J* = 2.1, 7.0 Hz). ^{13}C-NMR ($CDCl_3$): δ = 157.61, 157.59, 156.85, 156.83, 156.31, 155.33, 155.27, 139.28, 139.24, 130.11, 129.92, 129.76, 128.96, 128.93, 127.59, 127.55, 123.45, 123.44, 121.50, 121.47, 120.83, 119.11, 117.86, 117.05, 117.03, 111.55, 84.13, 84.04, 83.97, 82.50, 79.81, 79.76, 74.68, 74.61, 74.55, 69.37, 69.33, 69.28, 67.63, 67.59, 67.46, 67.41, 66.86, 63.55, 63.49, 63.37, 63.32, 54.43, 54.35, 33.87, 33.82, 29.76, 29.72, 29.67, 28.29, 27.91, 25.93, 25.88, 14.99. ^{31}P-NMR ($CDCl_3$): δ = -6.19, -6.53. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_{43}H_{60}NNaO_{13}P^+$: 852.3694. Found: 852.3736.

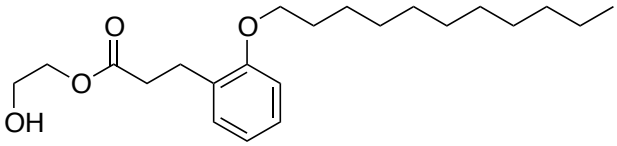
Synthesis of 14



TFA (0.6 mL) was added to **85** (38.5 mg, 0.046 mmol) at 0ºC. The solution was stirred for 1 h at room temperature, then diluted with CH_2Cl_2 (10 mL) and evaporated. The residue was chromatographed repeatedly: with $CHCl_3$:MeOH:AcOH = 9:1:0, 8:1:1 to 7:1:2, then $CHCl_3$:MeOH:H_2O = 9:1:0 to 65:25:4, then $CHCl_3$:MeOH:AcOH = 8:1:1 to 7:1:2 to yield **14** as the AcOH salt (13.8 mg, 0.022 mmol, 48%, white oil). The AcOH salt was dissolved in TFA and evaporated to yield **14** as the TFA salt (white powder). ^{1}H-NMR ($CDCl_3$/TFA-d): δ = 7.343 (3H, m), 7.194 (1H, m), 7.151-7.068 (4H, m), 7.004 (2H, m), 6.952 (1H, dd, *J* = 2.0, 8.0 Hz), 6.907 (2H, m), 5.081 (2H, s), 4.603 (3H, brs), 4.473 (1H, s), 4.335 (1H, dd, *J* = 3.2, 12 Hz), 4.190 (1H, dd, *J* = 2.6, 11.8 Hz), 3.712-3.576 (4H, m), 2.956 (2H, t, *J* = 7.2 Hz), 2.734 (2H, t, *J* = 7.2 Hz). ^{31}P-NMR ($CDCl_3$/TFA-d): δ = -2.49. HRMS (ESI-TOF, $[M-H]^-$): Calcd for $C_{30}H_{35}NO_{11}P^-$: 616.1953. Found: 616.2000. Mp: 159.0ºC-160.8 ºC, colorless cubes. Anal. Calcd. for $C_{30}H_{38}NO_{10}P$・1.7CF_3COOH: C, 49.44; H, 4.68; N, 1.73. Found: C, 49.15; H, 4.81; N, 1.80.

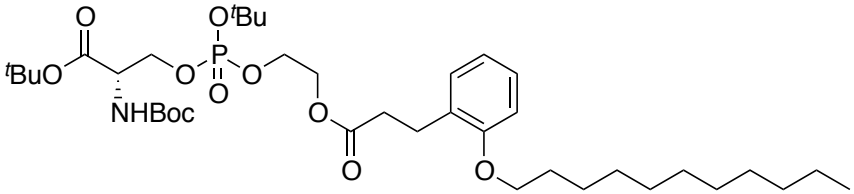
Synthesis of 15 (deoxy-LysoPS C2 C3-ph-o-O-C11: *O*-(hydroxy(2-((3-(2-(undecyloxy)phenyl)propanoyl)oxy)ethoxy)phosphoryl)-*L*-serine) (Figure 34)

Synthesis of 86



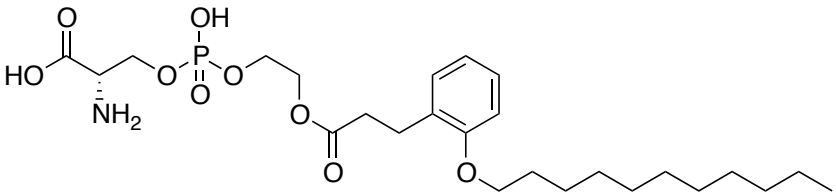
Ethylene glycol (1.00 mL, 1.11 g, 17.88 mmol) and carboxylic acid **C3-ph-o-O-C11 (A)** (249.5 mg, 0.779 mmol) were dissolved in anhydrous CH_2Cl_2 (4 mL). DMAP (23.3 mg, 0.191 mmol), and EDCI・HCl (318.2 mg, 1.660 mmol) were added to the solution, and the mixture was stirred at room temperature for 13 h. The reaction was quenched with water (20 mL) and this mixture was extracted three times with CH_2Cl_2 (20 mL). The combined organic layer was washed with brine (20 mL). The combined aqueous layer was extracted with CH_2Cl_2 (20 mL). The whole organic solution was dried over Na_2SO_4 and filtered. The filtrate was evaporated and the residue was chromatographed (n-hexane:AcOEt = 4:1 to 3:1) to yield **86** (246.1 mg, 0.675 mmol, 87%, colorless oil). ^{1}H-NMR ($CDCl_3$): δ = 7.167 (2H, m), 6.856 (2H, m), 4.180 (2H, m), 3.966 (2H, t, *J* = 6.6 Hz), 3.751 (2H, m), 2.965 (2H, t, *J* = 7.4 Hz), 2.683 (2H, m), 1.882 (1H, brs), 1.801 (2H, m), 1.471 (2H, m), 1.398-1.277 (14H, m), 0.888 (3H, t, *J* = 6.8 Hz). ^{13}C-NMR ($CDCl_3$): δ = 173.66, 156.95, 129.90, 128.71, 127.60, 120.17, 111.11, 67.84, 65.96, 61.21, 34.06, 31.87, 29.58, 29.56, 29.33, 29.30, 26.21, 26.11, 22.64, 14.06. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_{22}H_{36}NaO_4^+$: 387.2506. Found: 387.2506.

Synthesis of 87



The alcohol **86** (244.8 mg, 0.672 mmol) was dissolved in CH_2Cl_2 and co-evaporated with toluene. To the residue, **28** (194.9 mg, 0.420 mmol), CH_2Cl_2 and toluene was added, and the solvent was evaporated under vacuum. Under an argon atmosphere, the residue was dissolved in anhydrous CH_2Cl_2 (3 mL), and a solution of 1*H*-tetrazole (49.2 mg, 0.702 mmol) in anhydrous THF (3 mL) was added at room temperature. After a few minutes, a white solid precipitated. The reaction mixture was stirred for 4 h at room temperature, then diluted with saturated $NaHCO_3$ aq (10 ml), and the mixture was extracted three times with CH_2Cl_2 (10 ml). The combined organic layer was washed with brine (10 mL). The combined aqueous layer was extracted with CH_2Cl_2 (10 mL). The whole organic solution was dried over Na_2SO_4 and filtered. The filtrate was evaporated and the residue was chromatographed (n-hexane: AcOEt: Et_3N = 35:4:1). The solvent was evaporated and the residue was dissolved in anhydrous CH_2Cl_2 (6 mL). TBHP in decane (5.0-6.0 M) (130.6 μL, 0.653 mmol) was added, and the mixture was stirred for 5 h at room temperature under an argon atmosphere. The solvent was evaporated and the residue was chromatographed (n-hexane: AcOEt = 3:1) to yield **87** (207.1 mg, 0.278 mmol, 66 %, colorless oil). ^{1}H-NMR ($CDCl_3$): δ = 7.159 (2H, m), 6.843 (2H, m), 5.513 (1H, d, *J* = 8.0 Hz), 4.349 (2H, m), 4.278-4.087 (5H, m), 3.953 (2H, t, *J* = 6.6 Hz), 2.946 (2H, t, *J* = 7.6 Hz), 2.656 (2H, dt, *J* = 7.7, 2.9 Hz), 1.795 (2H, quintet, *J* = 7.0 Hz), 1.495-1.441 (29H, m), 1.394-1.271 (14H, m), 0.881 (3H, t, *J* = 6.8 Hz). ^{13}C-NMR ($CDCl_3$): δ = 172.90, 172.89, 168.19, 156.81, 155.10, 129.82, 128.57, 127.44, 120.05, 110.86, 83.90, 83.83, 82.56, 82.53, 79.79, 67.62, 67.45, 67.39, 67.34, 64.90, 64.84, 62.71, 62.70, 62.64, 62.62, 54.35, 54.27, 33.77, 31.78, 29.65, 29.63, 29.61, 29.59, 29.49, 29.46, 29.25, 29.21, 29.18, 28.18, 27.81, 26.00, 25.91, 22.55, 13.99. ^{31}P-NMR ($CDCl_3$): δ = -5.71, -5.86. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_{38}H_{66}NNaO_{11}P^+$: 766.4266. Found: 766.4246.

Synthesis of 15

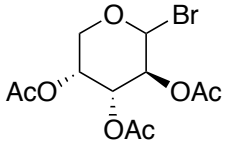


87 (205.7 mg, 0.277 mmol) was dissolved in 1,3-dimethoxybenzene (0.5 mL) and the solution was cooled to 0°C. TFA (2 mL) was added, and the mixture was stirred for 0.5 h at 0 ^{0}C and for 8 h at room temperature, then diluted with CH_2Cl_2 (10 mL) and evaporated. The residue was chromatographed ($CHCl_3$:MeOH:AcOH = 9:1:0, 8:1:1 to 7:1:2) to yield **15** as the AcOH salt (136.6 mg, 0.257 mmol, 93%, white solid). The AcOH salt was dissolved in TFA and evaporated to yield **15** as the TFA salt (white solid). ^{1}H-NMR ($CDCl_3$/TFA-d): δ = 7.214 (1H, m), 7.125 (1H, m), 6.897 (2H, m), 4.646 (2H, m), 4.535 (1H, brs), 4.371 (2H, t, *J* = 4.2 Hz), 4.248 (2H, m), 4.030 (2H, t, *J* = 6.8 Hz), 2.975 (2H, t, *J* = 7.4 Hz), 2.780 (2H, t, *J* = 7.6 Hz), 1.806 (2H, quintet, *J* = 7.1 Hz), 1.452 (2H, m), 1.375-1.280 (14H, m), 0.881 (3H, t, *J* = 6.6 Hz). ^{31}P-NMR ($CDCl_3$/TFA-d): δ = -1.33. HRMS (ESI-TOF, [M-H]$^-$): Calcd for $C_{25}H_{41}NO_9P^-$: 530.2524. Found: 530.2534. Mp: 129.0°C-133.5 °C, colorless cubes. Anal. Calcd. for $C_{25}H_{42}NO_9P \cdot 0.3CF_3COOH$: C, 54.34; H, 7.54; N, 2.48. Found: C, 54.05; H, 7.23; N, 2.40.

Synthesis of conformationally constrained cyclic LysoPS derivatives

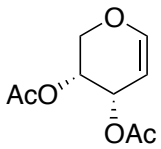
Synthesis of 18 (*O*-((((3*R*,4*S*)-4-((3-(2-(dodecyloxy)phenyl)propanoyl)oxy)-tetrahydro-2*H*-pyran-3-yl)oxy)(hydroxy)phosphoryl)-*L*-serine) and 22 (*O*-(hydroxy(((3*R*,4*S*)-3-((3-(2-(undecyloxy)phenyl)- propanoyl)oxy)tetrahydro-2*H*-pyran-4-yl)oxy)phosphoryl)-*L*-serine) (Figure 35)

Synthesis of 88[21]



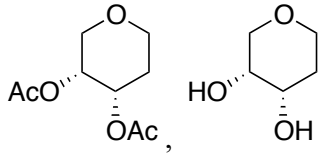
D-Arabinose (2.9888 g, 19.908 mmol) was dissolved in pyridine (30 mL) and the solution was cooled to 0°C. Acetic anhydride (16.3361 g, 160.017 mmol) was added, and the reaction mixture was stirred for 12 h at 0°C. Toluene (20 mL) was added and the mixture was evaporated three times. CH_2Cl_2 (150 mL) was added and the resulting mixture was extracted with 2 N HCl and 2 N NaOH, and washed with brine. The organic layer was dried over Na_2SO_4 and filtered. The filtrate was evaporated to give the corresponding tetraacetate (colorless oil). The crude product was directly used in the next step. A solution of HBr/AcOH (25 wt %, 12.9656 g, 40.062 mmmol) was added to the crude tetraacetate in anhydrous CH_2Cl_2 (20 mL) at 0°C. The mixture was stirred for 6 h at 0 to 5°C, then CH_2Cl_2 (100 mL) was added. The resulting mixture was extracted with ice-water (100 mL), iced sodium bicarbonate solution (100 mL) and ice-water (100 mL). The organic layer was dried over $CaCl_2$, and filtered, and the filtrate was evaporated. Et_2O was added to the residue and the mixture was kept at -25°C. Filtration and washing with Et_2O gave **acetobromo-D-arabinose, 88** (white solid, 5.0174 g, 14.794 mmol, 74 %, two steps). ^{1}H-NMR ($CDCl_3$): δ = 6.698 (1H, d, *J* = 3.6 Hz), 5.402 (2H, m), 5.087 (1H, m), 4.209 (1H, d, *J* = 13.6 Hz), 3.932 (1H, dd, *J* = 1.8, 13.4 Hz), 2.151 (3H, s), 2.111 (3H, s), 2.030 (3H, s). ^{13}C-NMR ($CDCl_3$): δ = 170.05, 170.01, 169.77, 89.64, 67.96, 67.84, 67.62, 64.70, 20.82, 20.73, 20.62. Mp: 134.5-136.5°C, white cubes.

Synthesis of 89



To a hot solution of cupric acetate monohydrate (639.5 mg, 3.203 mmol) in 20 mL of acetic acid was added zinc (11.2080 g, 171.429 mmol) at 70 to 80°C. The oil bath was removed, the solution was stirred for 90 seconds at room temperature, and the acetic acid was evaporated off. Acetic acid (4 mL) was added to the residue, and the mixture was stirred for 90 seconds, then the acetic acid was removed again. This was repeated another four times. Anhydrous Et_2O (10 mL) was added to the residue, and the mixture was stirred for 90 seconds, and the Et_2O was removed. This was repeated another two times to yield the zinc-copper couple. To the prepared zinc-copper couple, anhydrous THF (80 mL) and AcOH (4 mL) were added. AcONa (470.0 mg, 5.730 mmol) and acetobromo-D-arabinose **88** (2.0106 g, 5.929 mmol) were added at 0°C and the mixture was stirred for 2 h at 0°C and 13.5 h at room temperature, then filtered through Celite®. The Celite® was washed with CH_2Cl_2 (200 mL). The filtrate was extracted twice with saturated $NaHCO_3$ aq. (80 mL), and washed twice with brine (80 mL). The organic layer was dried over Na_2SO_4, and filtered. The filtrate was evaporated and the residue was chromatographed (n-hexane:AcOEt = 3:1) to yield **89** (colorless oil, 742.2 mg, 3.707 mmol, 63%). ^{1}H-NMR ($CDCl_3$): δ = 6.423 (1H, dd, *J* = 0.4, 6.0 Hz), 5.354 (1H, m), 5.098 (1H, m), 4.767 (1H, dd, *J* = 4.8, 6.0 Hz), 3.929 (2H, m), 1.991 (3H, s), 1.984 (3H, s). ^{13}C-NMR ($CDCl_3$): δ = 170.12, 169.52, 147.56, 97.29, 65.72, 62.80, 62.62, 20.76, 20.49. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_9H_{12}NaO_5^+$: 223.0577. Found: 223.0578.

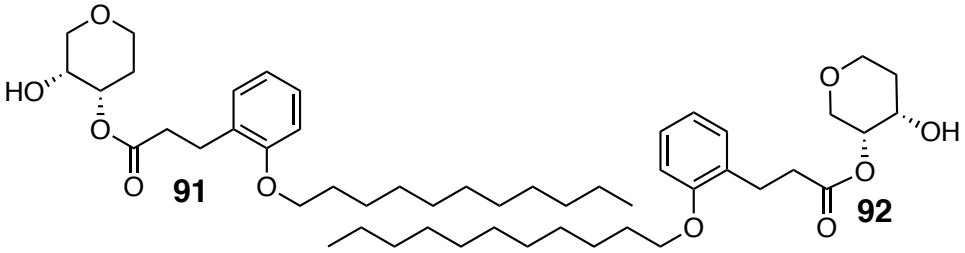
Synthesis of 90



89 (1.5322 g, 7.654 mmol) was dissolved in anhydrous MeOH (20 mL), and Pd-C (149.9 mg) in anhydrous methanol was added to the solution. Under an H_2 atmosphere, the reaction mixture was stirred for 4.5 h, then filtered through Celite®. The filtrate was evaporated, and the residue was chromatographed (n-hexane:AcOEt = 4:1) to yield **diacetate of 90** (colorless oil, 1.3723 g, 6.787 mmol, 89%). ^{1}H-NMR ($CDCl_3$): δ = 4.994 (2H, m), 3.804 (2H, m), 3.495 (2H, m), 2.005 (3H, s), 1.933 (4H, m), 1.708 (1H, m). ^{13}C-NMR ($CDCl_3$): δ = 169.99, 169.85, 68.29, 67.74, 67.04 64.75, 27.68, 20.70, 20.68. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_9H_{14}NaO_5^+$: 225.0733. Found: 225.0720.

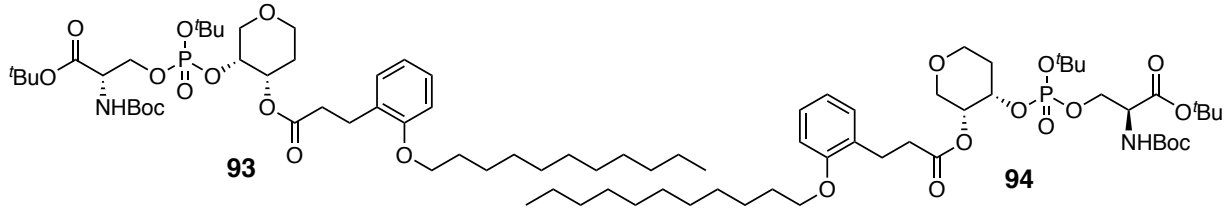
Diacetate of 90 (1.3651 g, 6.751 mmol) was dissolved in anhydrous MeOH (15 mL) and the solution was cooled to 0°C. Sodium methoxide (185.3 mg, 3.430 mmol) was added at 0°C, and the reaction mixture was stirred for 2 h at room temperature. Under an argon atmosphere, the solution was evaporated to remove the MeOH, and the residue was chromatographed (AcOEt to $CHCl_3$: MeOH = 9:1) to yield **90** (white solid, 763.2 mg, 6.461 mmol, 91%). ^{1}H-NMR ($CDCl_3$): δ = 3.833 (3H, m), 3.752 (1H, m), 3.516 (1H, dd, *J* = 2.4, 11.6 Hz), 3.429 (1H, m), 3.106-2.278 (2H, m), 1.847 (1H, m), 1.753 (1H, m). ^{13}C-NMR ($CDCl_3$): δ = 69.34, 68.23, 67.88, 64.82, 30.48.

Synthesis of 91 and 92



A mixture of diol **90** (80.9 mg, 0.685 mmol), **C3-ph-o-O-C11 (B)** (190.6 mg, 0.595 mmol), DMAP (15.7 mg, 0.129 mmol), and EDCI・HCl (187.7 mg, 0.979 mmol) in CH_2Cl_2 (6 mL) was stirred for 5.5 h under an argon atmosphere. The reaction was quenched with water (15 mL) and the mixture was extracted three times with CH_2Cl_2 (15 mL). The combined organic layer was washed with brine. The combined aqueous layer was extracted with CH_2Cl_2 (15 mL). The whole organic solution was dried over Na_2SO_4 and filtered. The filtrate was evaporated and the residue was chromatographed (n-hexane:AcOEt = 4:1 to 2:1) to yield a mixture of **91** and **92** (182.3 mg, 0.433 mmol, 73%, colorless oil). This mixture was directly used in the next reaction without separation of the regioisomers. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_{25}H_{40}NaO_5^+$:443.2768. Found: 443.2724.

Synthesis of 93 and 94



The mixture of **91** and **92** (136.6 mg, 0.325 mmol) and **28** (208.4 mg, 0.449 mmol) were dissolved in CH_2Cl_2 and co-evaporated with toluene. Under an argon atmosphere, the residue was dissolved in anhydrous CH_2Cl_2 (3 mL), and a solution of 1*H*-tetrazole (47.0 mg, 0.671 mmol) in anhydrous THF (3 mL) was added at room temperature. After a few minutes, a white solid precipitated. The reaction mixture was stirred for 4 h at room temperature, then diluted with saturated $NaHCO_3$ aq (10 ml) and extracted three times with CH_2Cl_2 (10 ml). The combined organic layer was washed with brine (10 mL). The combined aqueous layer was extracted with CH_2Cl_2 (10 mL). The whole organic solution was dried over Na_2SO_4 and filtered. The filtrate was evaporated and the residue was chromatographed (n-hexane:AcOEt:Et_3N = 35:4:1). The residue was dissolved in anhydrous CH_2Cl_2 (10 mL). TBHP in decane (5.0-6.0 M) (136.5 μL, 0.683 mmol) was added, and the mixture was stirred for 5 h at room temperature under an argon atmosphere. The solution was diluted with water (20 mL) and extracted three times with CH_2Cl_2 (20 mL). The combined organic layer was washed with brine (20 mL). The combined aqueous layer was extracted with CH_2Cl_2 (10 mL). The whole organic solution was dried over Na_2SO_4 and filtered. The filtrate was evaporated and the residue was chromatographed (n-hexane:AcOEt = 2:1) several times to yield **93** (54.3 mg, 0.068 mmol, 21 %, colorless oil) and **94** (47.9 mg, 0.060 mmol, 18%, thick oil).

93

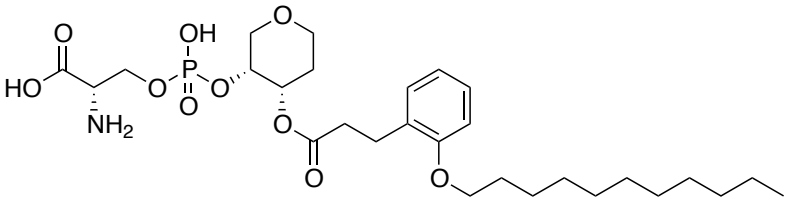
^{1}H-NMR ($CDCl_3$): δ = 7.147 (2H, m), 6.825 (2H, m), 5.693 (1H, m), 5.072 (1H, m), 4.498 (1H, m), 4.351 (2H, m), 4.230 (1H, m), 3.949 (3H, m), 3.792 (1H, m), 3.615 (1H, ddd, *J* = 2.3, 12.3, 18.9 Hz), 3.516 (1H, m), 2.952 (2H, m), 2.667 (2H, m), 1.981 (1H, m), 1.847-1.691 (3H, m), 1.461 (29H, m), 1.361-1.231 (14H, m), 0.875 (3H, t, *J* = 6.8 Hz). ^{13}C-NMR ($CDCl_3$): δ = 172.64, 172.45, 168.39, 168.35, 156.96, 155.33, 129.95, 129.93, 128.73, 128.69, 127.57, 127.51, 120.17, 111.05, 111.04, 84.14, 84.07, 82.58, 82.51, 79.81, 72.26, 72.21, 72.16, 69.04, 68.97, 68.89, 68.83, 68.05, 67.99, 67.81, 67.75, 67.38, 64.71, 64.49, 54.49, 54.42, 34.13, 34.08, 31.90, 29.81, 29.78, 29.76, 29.74, 29.63, 29.61, 29.60, 29.38, 29.33, 28.32, 28.03, 27.94, 27.86, 26.13, 26.05, 26.01, 22.66, 14.09. ^{31}P-NMR ($CDCl_3$): δ = -6.20, -6.39. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_{36}H_{58}NNaO_9PSi^+$: 822.4528. Found: 822.4551.

94

^{1}H-NMR ($CDCl_3$): δ = 7.158 (2H, m), 6.837 (2H, m), 5.573 (1H, m), 5.032 (1H, m), 4.648 (1H, m), 4.333 (2H, m), 4.215 (1H, m), 3.952 (2H, t, *J* = 6.6 Hz), 3.852 (2H, m), 3.550 (2H, m), 2.956 (2H, m), 2.689 (2H, m), 2.128 (1H, m), 1.794 (2H, quintet, *J* = 7.0 Hz), 1.460 (29H, m), 1.363-1.255 (14H, m), 0.880 (3H, t, *J* = 7.0 Hz). ^{13}C-NMR ($CDCl_3$): δ = 172.53, 172.46, 168.43, 168.30, 156.92, 155.31, 155.23, 129.94, 129.92, 128.74, 127.48, 120.15, 110.99, 84.05, 83.98, 82.60, 82.55, 79.93, 79.86, 72.92, 72.87, 72.67, 72.62, 68.51, 68.45, 68.43, 68.37, 67.78, 67.52, 67.48, 67.44, 66.64, 66.62, 66.39, 64.25, 64.08, 54.50, 54.44, 54.36, 34.05, 31.87, 29.82, 29.78, 29.76, 29.75, 29.72, 29.60, 29.58, 29.57, 29.36, 29.30, 28.29, 27.93, 26.08, 25.90, 22.64, 14.07. ^{31}P-

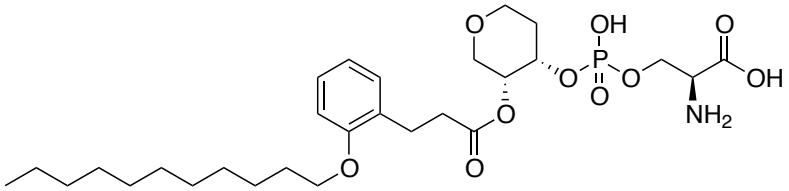
NMR ($CDCl_3$): δ = -5.99 -6.19. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_{36}H_{58}NNaO_9PSi^+$: 822.4528. Found: 822.4509.

Synthesis of 18



TFA (1.5 mL) was added to **93** (54.5 mg, 0.068 mmol) at 0°C, and the mixture was stirred for 1 h at room temperature. The reaction mixture was diluted with CH_2Cl_2 (10 mL) and the solution was evaporated. The residue was chromatographed ($CHCl_3$:MeOH:AcOH = 8:1:1, 7:1:2 to 6:1:3) to yield **18** as the AcOH salt (37.1 mg, 0.063 mmol, 92%, white solid). The AcOH salt was dissolved in TFA and evaporated to yield **18** as the TFA salt (white solid). ^{1}H-NMR ($CDCl_3$/TFA-d): δ = 7.202 (1H, m), 7.098 (1H, m), 6.883 (2H, m), 5.239 (1H, brs), 4.614 (3H, m), 4.513 (1H, brs), 4.017 (2H, t, *J* = 6.8 Hz), 3.966-3.724 (4H, m), 2.979 (2H, m), 2.797 (2H, m), 1.885 (2H, m), 1.800 (2H, quintet, *J* = 7.1 Hz), 1.444 (2H, m), 1.369-1.227 (14H, m), 0.879 (3H, t, *J* = 6.6 Hz). ^{31}P-NMR ($CDCl_3$/TFA-d): δ = -2.41. HRMS (ESI-TOF, $[M-H]^-$): Calcd for $C_{28}H_{45}NO_{10}P^-$: 586.2787. Found: 586.2813. Mp: 124.0°C-126.8°C, white powder. Anal. Calcd. for $C_{28}H_{46}NO_{10}P \cdot 0.7CF_3COOH$: C, 52.90; H, 7.05; N, 2.10. Found: C, 53.06; H, 7.26; N, 1.95.

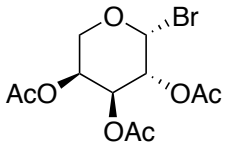
Synthesis of 22



TFA (1.5 mL) was added to **94** (55.5 mg, 0.069 mmol) at 0°C, and the mixture was stirred for 2.5 h at room temperature, then diluted with CH_2Cl_2 (10 mL) and evaporated. The residue was chromatographed ($CHCl_3$/MeOH/AcOH = 8:1:1, 7:1:2 to 6:1:3) to yield **22** as the AcOH salt (37.6 mg, 0.064 mmol, 93%, white solid). The AcOH salt was dissolved in TFA and evaporated to yield **22** as the TFA salt (white solid). ^{1}H-NMR ($CDCl_3$/TFA-d): δ = 7.213 (1H, m), 7.112 (1H, m), 6.887 (2H, m), 5.232 (1H, brs), 4.709-4.436 (4H, m), 4.163 (1H, d, *J* = 11.6 Hz), 4.020 (2H, t, *J* = 6.8 Hz), 3.957 (1H, d, *J* = 11.6 Hz), 3.682 (2H, m), 2.974 (2H, t, *J* = 6.6 Hz), 2.818 (2H, t, *J* = 6.4 Hz), 2.227 (1H, m), 1.961 (1H, d, *J* = 12 Hz), 1.797 (2H, quintet, *J* = 6.9 Hz), 1.437 (2H, m), 1.365-1.225 (14H, m), 0.878 (3H, t, *J* = 6.8 Hz). ^{31}P-NMR ($CDCl_3$/TFA-d): δ = -2.38. HRMS (ESI-TOF, $[M-H]^-$): Calcd for $C_{28}H_{45}NO_{10}P^-$: 586.2787. Found: 586.2827. Mp: 134.0°C-136.0°C, white cubes. Anal. Calcd. for $C_{28}H_{46}NO_{10}P \cdot 0.7CF_3COOH$: C, 52.90; H, 7.05; N, 2.10. Found: C, 52.68; H, 7.12; N, 1.94.

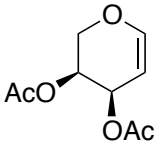
Synthesis of cyclic glycol derivatives 19 (*O*-(hydroxy(((3*S*,4*R*)-4-((3-(2-(undecyloxy)phenyl)propanoyl)oxy)tetrahydro-2*H*-pyran-3-yl)oxy)phosphoryl)-*L*-serine) and 24 (*O*-(hydroxy(((3*S*,4*R*)-3-((3-(2-(undecyloxy)phenyl)propanoyl)oxy)tetrahydro-2*H*-pyran-4-yl)oxy)phosphoryl)-*L*-serine) (Figure 36)

Synthesis of 95



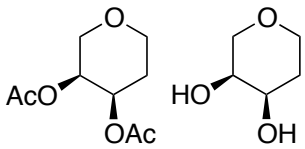
L-Arabinose (3.0004 g, 19.985 mmol) was dissolved in pyridine (30 mL) and the solution was cooled to 0°C. Acetic anhydride (16.3431 g, 160.085 mmol) was added, and the reaction mixture was stirred for 28 h at 0 to 15°C. Toluene was added, and the mixture was evaporated. CH_2Cl_2 (120 mL) was added to the residue, and the mixture was extracted with sat. $NaHCO_3$ (60 mL), 2 N HCl (60 mL), 2 N NaOH (40 mL), and washed with brine (50 mL). The organic layer was dried over Na_2SO_4, and filtered. The filtrate was evaporated. Et_2O (120 mL) was added to the residue, and the mixture was extracted with 5% $KHSO_4$ 6 times (total 230 mL). The combined aqueous layer was extracted with Et_2O three times (30 mL). The combined organic layer was washed with brine, dried over Na_2SO_4 and filtered. The filtrate was evaporated to give the corresponding tetraacetate (yellow oil, 6.3704 g). The crude product was directly used in the next step. A solution of HBr/AcOH (25 wt %, 12.1162 g, 37.437 mmmol) was added to the crude tetraacetate (5.9553 g) in anhydrous CH_2Cl_2 (20 mL) at 0°C. The mixture was stirred for 1.5 h at 0°C, and then CH_2Cl_2 (100 mL) was added. The resulting mixture was extracted with ice-water (100 mL), iced sodium bicarbonate solution (100 mL) and ice-water (100 mL). The organic layer was dried over $CaCl_2$, and filtered. The filtrate was evaporated. Et_2O was added to the residue at -25°C. Filtration and washing with Et_2O gave **acetobromo-L-arabinose, 95** (white solid, 3.9362 g, 11.606 mmol, 62 %, two steps). ^{1}H-NMR ($CDCl_3$): δ = 6.694 (1H, d, *J* = 3.6 Hz), 5.396 (2H, m), 5.082 (1H, dd, *J* = 1.6, 4.0 Hz), 4.205 (1H, d, *J* = 13.2 Hz), 3.928 (1H, dd, *J* = 1.6, 13.6 Hz), 2.147 (3H, s), 2.107 (3H, s), 2.025 (3H, s). ^{13}C-NMR ($CDCl_3$): δ = 170.05, 170.01, 169.77, 89.63, 67.95, 67.83, 67.60, 64.69, 20.82, 20.73, 20.62. Mp: 142.0-143.5°C, white solid.

Synthesis of cyclic 96



To a solution of cupric acetate monohydrate (638.6 mg, 3.199 mmol) in 20 mL of acetic acid was added zinc (11.2053 g, 171.309 mmol) at room temperature. The solution was stirred for 90 seconds and the acetic acid was removed by evaporation. Acetic acid (20 mL) was added to the residue, and the mixture was stirred for 90 seconds, and then the acetic acid was removed. This was repeated another four times. Anhydrous Et_2O (40 mL) was added to the residue, and the mixture was stirred for 90 seconds, and then the Et_2O removed. This was repeated another two times to yield zinc-copper couple. To the prepared zinc-copper couple, anhydrous THF (80 mL) and AcOH (4 mL) were added. NaOAc (560.6 mg, 6.103 mmol) and acetobromo-L-arabinose **95** (2.0053 g, 5.913 mmol) were then added. The mixture was stirred for 16 h at room temperature, and filtered through Celite®. The filtrate was washed with CH_2Cl_2 and extracted three times with saturated $NaHCO_3$ (50 mL), then washed twice with brine (total 150 mL). The organic layer was dried over Na_2SO_4, and filtered. The filtrate was evaporated. The remaining aqueous solution was extracted three times with CH_2Cl_2 (30 mL) and washed with brine (20 mL). The combined aqueous layer was extracted with CH_2Cl_2 (20 mL). The whole organic solution was dried over Na_2SO_4 and filtered. The filtrate was evaporated and the residue was chromatographed (n-hexane:AcOEt = 1:0, 4:1 to 3:1) to yield **96** (colorless oil, 566.7 mg, 2.831 mmol, 48%). ^{1}H-NMR ($CDCl_3$): δ = 6.431 (1H, dd, *J* = 0.4, 6.0 Hz), 5.362 (1H, m), 5.106 (1H, m), 4.775 (1H, dd, *J* = 5.2, 6.0 Hz), 3.938 (2H, m), 2.000 (3H, s), 1.993 (3H, s). ^{13}C-NMR ($CDCl_3$): δ = 170.15, 169.55, 147.57, 97.30, 65.73, 62.63, 20.79, 20.52. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_9H_{12}NaO_5^+$: 223.0577. Found: 223.0613.

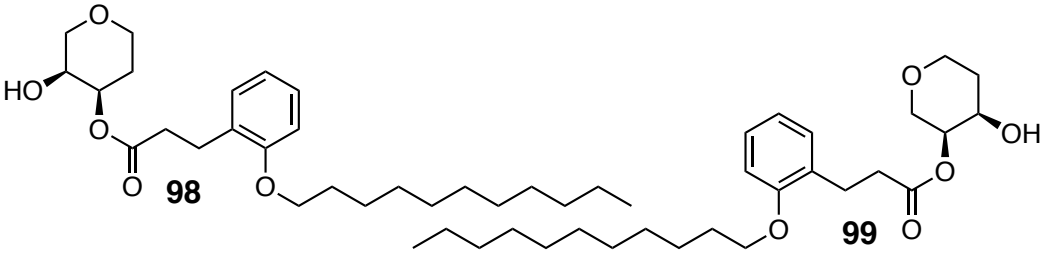
Synthesis of 97



96 (1.6056 g, 8.020 mmol) was dissolved in anhydrous MeOH (20 mL), and Pd-C (159.2 mg) in anhydrous methanol (18 mL) was added to the solution. Under an H_2 atmosphere, the mixture was stirred for 4 h, then filtered through Celite®. The filtrate was evaporated, and the residue was chromatographed (n-hexane:AcOEt = 4:1) to yield **diacetate of 97** (colorless oil, 1.5186 g, 7.510 mmol, 94%). ^{1}H-NMR ($CDCl_3$): δ = 5.044 (2H, m), 3.854 (2H, m), 3.546 (2H, m), 2.060 (3H, s), 1.992 (4H, m), 1.758 (1H, m). ^{13}C-NMR ($CDCl_3$): δ = 170.17, 170,04, 68.42, 67.85, 67.20 64.91, 27.79, 20.85, 20.83. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_9H_{14}NaO_5^+$: 225.0733. Found: 225.0721.

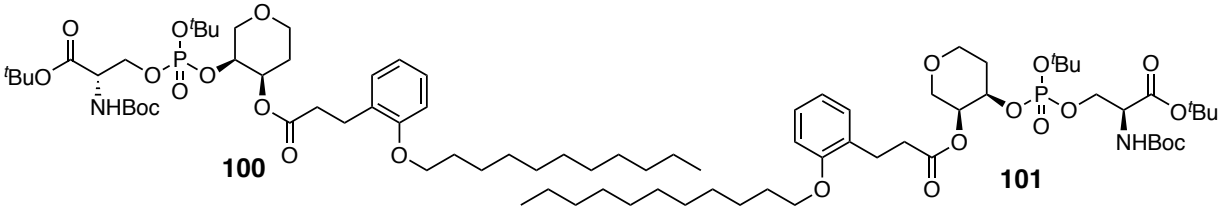
Diacetate of 97 (1.5071 g, 7.454 mmol) was dissolved in anhydrous MeOH (15 mL) and the solution was cooled to 0ºC. Sodium methoxide (201.6 mg, 3.732 mmol) was added at 0ºC under an argon atmosphere, and the reaction mixture was stirred for 5.5 h at room temperature. The solution was evaporated to remove MeOH and the residue was chromatographed (AcOEt to $CHCl_3$: MeOH = 9:1) to yield **97** (colorless oil, 805.1 mg, 6.815 mmol, 91%). ^{1}H-NMR ($CDCl_3$): δ = 3.766 (5H, m), 3.685 (1H, m), 3.455 (1H, dd, *J* = 2.4 Hz, 11.6 Hz), 3.379 (1H, m), 1.812 (1H, m), 1.673 (1H, m). ^{13}C-NMR ($CDCl_3$): δ = 69.11, 68.07, 67.74, 64.66, 30.17.

Synthesis of 98 and 99



To a solution of diol **97** (122.5 mg, 1.037 mmol), DMAP (13.4 mg, 0.110 mmol), and EDCI・HCl (214.0 mg, 1.116 mmol) in CH_2Cl_2 (10 mL) were added carboxylic acid **C3-ph-o-O-C11 (B)** (243.9 mg, 0.761 mmol) and CH_2Cl_2 (2 mL) at 0ºC. The mixture was stirred for 0.5 h at 0ºC and for 4.5 h at room temperature under an argon atmosphere. The reaction was quenched with water (20 mL) and the mixture was extracted three times with CH_2Cl_2 (10 mL). The combined organic layer was washed with brine (10 mL). The combined aqueous layer was extracted with CH_2Cl_2 (10 mL). The whole organic solution was dried over Na_2SO_4 and filtered. The filtrate was evaporated and the residue was chromatographed (n-hexane:AcOEt = 4:1 to 3:1) to yield a mixture of **98** and **99** (265.5 mg, 0.631 mmol, 83%, colorless oil). This mixture was directly used in the next reaction without separation of the regioisomers. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_{25}H_{40}NaO_5^+$: 443.2768. Found: 443.2761.

Synthesis of 100 and 101



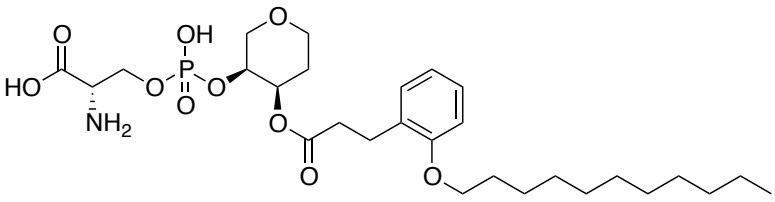
A mixture of **98** and **99** (265.6 mg, 0.631 mmol) was dissolved in CH_2Cl_2 and co-evaporated with toluene. **28** (440.1 mg, 0.947 mmol) was added, and the mixture was dissolved in CH_2Cl_2 and co-evaporated with toluene. Under an argon atmosphere, the residue was dissolved in anhydrous CH_2Cl_2 (5 mL), and to this solution, a solution of 1*H*-tetrazole (88.4 mg, 1.262 mmol) in anhydrous THF (5 mL) was added at room temperature. After a few minutes, a white solid precipitated. The reaction mixture was stirred for 3 h at room temperature, diluted with saturated

$NaHCO_3$ aq. (16 ml), and extracted three times with CH_2Cl_2 (10 ml). The combined organic layer was washed with brine (10 mL). The combined aqueous layer was extracted with CH_2Cl_2 (10 mL). The whole organic solution was dried over Na_2SO_4 and filtered. The filtrate was evaporated and the residue was chromatographed (n-hexane:AcOEt:Et_3N = 35:4:1). The residue was dissolved in anhydrous CH_2Cl_2 (10 mL). TBHP in decane (5.0-6.0 M) (214 μL, 0.1.070 mmol) was added to the solution, and the mixture was stirred for 12.5 h at room temperature under an argon atmosphere. The solution was diluted with water (20 mL) and extracted three times with CH_2Cl_2 (20 mL). The combined organic layer was washed with brine (20 mL). The combined aqueous layer was extracted twice with CH_2Cl_2 (total 30 mL). The whole organic solution was dried over Na_2SO_4 and filtered. The filtrate was evaporated and the residue was chromatographed (n-hexane:AcOEt = 2:1) several times to yield **100** (149.6 mg, 0.187 mmol, 30%, colorless sticky solid) and **101** (98.5 mg, 0.123 mmol, 19%, thick oil).

100: ^{1}H-NMR ($CDCl_3$): δ = 7.151 (2H, m), 6.829 (2H, m), 5.612 (1H, m), 4.490 (1H, m), 4.349 (2H, m), 4.237 (1H, m), 3.922 (3H, m), 3.740 (1H, m), 3.639 (1H, m), 3.519 (1H, m), 2.959 (2H, dt, *J* = 2.9 Hz, 7.5 Hz,) 2.678 (2H, m), 1.947 (1H, m), 1.767 (3H, m), 1.458 (29H, m), 1.389-1.265 (14H, m), 0.877 (3H, t, *J* = 6.8 Hz). ^{13}C-NMR ($CDCl_3$): δ = 172.51, 172.44, 168.41, 168.36, 156.95, 155.33, 129.92, 128.70, 127.55, 127.51, 120.16, 111.02, 84.16, 84.12, 84.10, 84.08, 84.04, 83.98, 82.56, 79.82, 72.18, 72.13, 72.09, 72.04, 68.88, 68.81, 68.70, 68.64, 67.95, 67.79, 67.65, 67.60, 67.57, 67.54, 67.34, 67.33, 67.31, 67.28, 64.52, 64.49, 64.47, 64.27, 64.26, 54.54, 54.48, 54.46, 54.39, 34.14, 34.07, 31.89, 29.78, 29.74, 29.62, 29.60, 29.38, 29.32, 28.31, 27.93, 26.12, 26.04, 25.99, 25.59, 22.66, 14.09. ^{31}P-NMR ($CDCl_3$): δ = -6.11, -6.22. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_{36}H_{58}NNaO_9PSi^+$: 822.4528. Found: 822.4552.

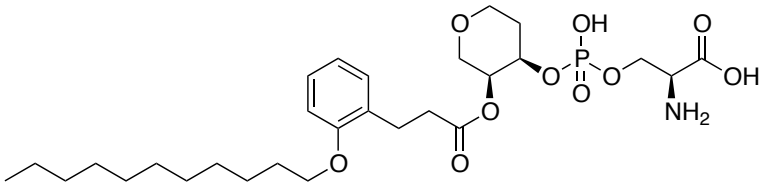
101: ^{1}H-NMR ($CDCl_3$): δ = 7.155 (2H, m), 6.841 (2H, m), 5.513 (1H, m), 5.012 (1H, m), 4.659 (1H, m), 4.328 (2H, m), 4.200 (1H, m), 3.951 (2H, t, *J* = 6.6 Hz), 3.844 (2H, m), 3.548 (2H, m), 2.954 (2H, m), 2.679 (2H, m), 2.123 (1H, m), 1.900 (1H, m), 1.791 (2H, quintet, *J* = 6.9 Hz), 1.455 (29H, m), 1.392-1.266 (14H, m), 0.878 (3H, t, *J* = 6.8 Hz). ^{13}C-NMR ($CDCl_3$): δ = 172.64, 172.48, 168.34, 168.31, 156.94, 155.29, 155.20, 129.98, 129.93, 128.79, 128.74, 128.70, 127.51, 127.47, 120.17, 120.15, 110.01, 111.00, 84.10, 84.05, 84.03, 83.98, 82.67, 82.60, 82.56, 79.97, 79.91, 72.83, 72.79, 72.77, 72.60, 72.55, 68.53, 68.48, 68.42, 67.79, 67.57, 67.54, 67.52, 66.66, 66.64, 66.61, 66.59, 66.35, 64.31, 64.28, 64.24, 64.12, 64.10, 64.06, 54.55, 54.49, 54.44, 54.36, 34.14, 34.05, 31.89, 29.82, 29.78, 29.76, 29.72, 29.62, 29.60, 29.59, 29.38, 29.32, 28.31, 27.96, 27.94, 26.10, 25.93, 22.66, 14.09. ^{31}P-NMR ($CDCl_3$): δ = -5.83 -6.25. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_{36}H_{58}NNaO_9PSi^+$: 822.4528. Found: 822.4482.

Synthesis of 19



TFA (1 mL) was added to **100** (48.4 mg, 0.061 mmol) at 0°C, and the reaction mixture was stirred for 1 h at room temperature, then diluted with CH_2Cl_2 (10 mL) and evaporated. The residue was chromatographed ($CHCl_3$:MeOH:AcOH = 8:1:1, 7:1:2 to 6:1:3) to yield **19** as the AcOH salt (34.8 mg, 0.059 mmol, 97%, colorless solid). The AcOH salt was dissolved in TFA and evaporated to yield **19** as the TFA salt (white solid). ^{1}H-NMR ($CDCl_3$/TFA-d): δ = 7.208 (1H, m), 7.076 (1H, m), 6.858 (2H, m), 5.276 (1H, brs), 4.555-4.462 (4H, m), 4.000 (2H, t, *J* = 6.6 Hz), 3.853 (2H, brs), 3.758 (2H, brs), 2.964 (2H, m), 2.788 (2H, m), 1.795 (4H, m), 1.441 (2H, m), 1.359-1.271 (14H, m), 0.877 (3H, t, *J* = 6.8 Hz). ^{31}P-NMR ($CDCl_3$/TFA-d): δ = -2.64. HRMS (ESI-TOF, $[M-H]^-$): Calcd for $C_{28}H_{45}NO_{10}P^-$: 586.2787. Found: 586.2864. Mp: 152.0°C-156.5°C, colorless cubes. Anal. Calcd. for $C_{28}H_{46}NO_{10}P \cdot 1.2CF_3COOH$: C, 50.40; H, 6.57; N, 1.93. Found: C, 50.45; H, 6.82; N, 1.99.

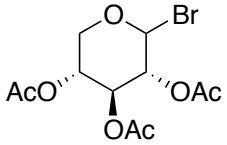
Synthesis of 24



TFA (1.5 mL) was added to **101** (57.1 mg, 0.071 mmol) at 0ºC, and the reaction mixture was stirred for 1 h at room temperature. The reaction mixture was diluted with CH_2Cl_2 (10 mL) and the solution was evaporated. The residue was chromatographed ($CHCl_3$/MeOH/AcOH = 8:1:1, 7:1:2 to 6:1:3) twice to yield **24** as the AcOH salt (26.6 mg, 0.045 mmol, 63%, white solid). The AcOH salt was dissolved in TFA and evaporated to yield **24** as the TFA salt (orange solid). ^{1}H-NMR ($CDCl_3$/TFA-d): δ = 7.212 (1H, m), 7.095 (1H, m), 6.874 (2H, m), 5.238 (1H, m), 4.588 (2H, m), 4.450 (1H, brs), 4.135 (1H, d, *J* = 12 Hz), 4.003 (2H, t, *J* = 6.8 Hz), 3.924 (1H, dd, *J* = 3.0, 13.0 Hz), 3.766-3.612 (2H, m), 2.967 (2H, t, *J* = 7.0 Hz), 2.797 (2H, m), 2.182 (1H, m), 1.924 (1H, d, *J* = 11.6 Hz), 1.788 (2H, quintet, *J* = 7.1 Hz), 1.431 (2H, m), 1.357-1.225 (14H, m), 0.878 (3H, t, *J* = 6.6 Hz). ^{31}P-NMR ($CDCl_3$/TFA-d): δ = -2.70. HRMS (ESI-TOF, $[M-H]^-$): Calcd for $C_{28}H_{45}NO_{10}P^-$: 586.2787. Found: 586.2775. Mp: 138.0ºC-140.5ºC, white cubes. Anal. Calcd. for $C_{28}H_{46}NO_{10}P \cdot 0.7CF_3COOH$: C, 52.90; H, 7.05; N, 2.10. Found: C, 52.55; H, 7.09; N, 2.11.

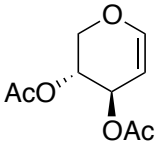
Synthesis of 20 (*O*-(hydroxy(((3*R*,4*R*)-4-((3-(2-(undecyloxy)phenyl)-propanoyl)oxy)tetrahydro-2*H*-pyran-3-yl)oxy)phosphoryl)-*L*-serine) and 23 (*O*-(hydroxy(((3*R*,4*R*)-3-((3-(2-(undecyloxy)phenyl)propanoyl)oxy)tetrahydro-2*H*-pyran-4-yl)oxy)phosphoryl)-*L*-serine). (Figure 37)

Synthesis of 102



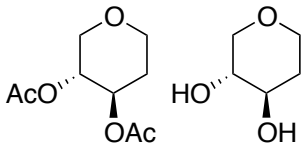
D-Xylose (2.9992 g, 19.977 mmol) was dissolved in pyridine (30 mL) and the solution was cooled to 0ºC. Acetic anhydride (16.3281 g, 159.938 mmol) was added, and the reaction mixture was stirred for 2 h at 0ºC and for 10 h at room temperature. Toluene was added, and the mixture was evaporated three times (total 80 mL). CH_2Cl_2 (150 mL) was added and the mixture was extracted with 2 N HCl (100 mL) and 2 N NaOH (100 mL), and washed with brine (100 mL). The organic layer was dried over Na_2SO_4. After filtration, the filtrate was evaporated to give **1,2,3,4-tetra-*O*-acetyl-D-xylopyranose**. The crude product was directly used in the next step without further purification. A solution of HBr/AcOH (25 wt %, 12.9840 g, 40.119 mmmol) was added to the crude tetraacetate in anhydrous CH_2Cl_2 (20 mL) at 0ºC. The mixture was stirred for 2.5 h at 0ºC and for 3 h at room temperature, then CH_2Cl_2 (100 mL) was added. The resulting mixture was extracted with iced sodium bicarbonate solution (100 mL) and twice with ice-water (100 mL). The organic layer was dried over $CaCl_2$, and filtered. The filtrate was evaporated, and Et_2O was added to the residue at -25ºC. Filtration and washing with Et_2O gave **2,3,4-tri-*O*-acetyl-α-bromo-D-xylopyranose 102** (green solid, 5.0771 g, 14.971 mmol, 75 %, two steps). ^{1}H-NMR ($CDCl_3$): δ = 6.571 (1H, d, *J* = 4.0 Hz), 5.553 (1H, t, *J* = 9.6 Hz), 5.031 (1H, m), 4.764 (1H, dd, *J* = 4.0, 9.6 Hz), 4.042 (1H, dd, *J* = 6.0, 11.2 Hz), 3.870 (1H, m), 2.089 (3H, s), 2.047 (6H, m). ^{13}C-NMR ($CDCl_3$): δ = 169.81, 169.71, 87.56, 70.85, 69.52, 68.07, 62.51, 20.62, 20.59. Mp: 93.0-94.0°C, white solid.

Synthesis of 103



To a hot solution of cupric acetate monohydrate (642.2 mg, 5.367 mmol) in 20 mL of acetic acid was added zinc (11.2129 g, 171.504 mmol) at 90 to 100ºC. The oil bath was removed, then the solution was stirred for 90 seconds at room temperature and the acetic acid was removed by evaporation. Acetic acid (5 mL) was added to the residue, and the mixture was stirred for 90 seconds, then the acetic acid was removed. This procedure was repeated another four times. Anhydrous Et_2O (4 mL) was added to the residue, and the mixture was stirred for 90 seconds, then the Et_2O was removed. This procedure was repeated another two times to yield zinc-copper couple. To the prepared zinc-copper couple, anhydrous THF (80 mL) and AcOH (4 mL) were added. AcONa (968.1 mg, 11.802 mmol) and **102** (2.0461 g, 6.033 mmol) were added at 0ºC, and the mixture was stirred for 30 minutes. Anhydrous THF (10 mL) was added and stirring was continued for 5.5 h at room temperature. The reaction mixture was filtered through Celite®. The filtrate was washed with CH_2Cl_2 (200 mL), extracted twice with saturated $NaHCO_3$ (100 mL), and washed with brine (100 mL). The combined aqueous layer was extracted three times with CH_2Cl_2 (50 mL). The whole organic solution was dried over Na_2SO_4, and filtered. The filtrate was evaporated and the residue was chromatographed (n-hexane:AcOEt = 3:1 to 2:1) to yield **103** (colorless oil, 948.9 mg, 4.740 mmol, 79%). ^{1}H-NMR ($CDCl_3$): δ = 6.543 (1H, d, *J* = 6.0 Hz), 4.914 (3H, m), 4.137 (1H, m), 3.920 (1H, dd, *J* = 1.8, 12.2Hz), 2.039 (3H, s), 2.009 (3H, s). ^{13}C-NMR ($CDCl_3$): δ = 169.70, 169.59, 147.87, 97.25, 67.05, 63.46, 63.27, 20,92, 20.74. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_9H_{12}NaO_5^+$: 223.0577. Found: 223.0585.

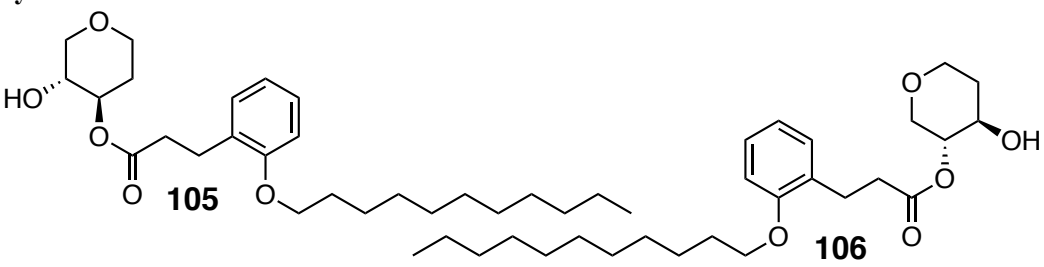
Synthesis of 104



103 (635.0 mg, 3.172 mmol) was dissolved in anhydrous MeOH (5 mL), and Pd-C (65.6 mg) in anhydrous methanol (5 mL) was added to the solution. Under an H_2 atmosphere, the mixture was stirred for 5.5 h, and then filtered through Celite®. The filtrate was evaporated, and the residue was chromatographed (n-hexane:AcOEt = 3:1 to 2:1) to yield **diacetate of 104** (colorless oil, 500.8 mg, 2.477 mmol, 78%). ^{1}H-NMR ($CDCl_3$): δ = 5.044 (2H, m), 3.854 (2H, m), 3.546 (2H, m), 2.060 (3H, s), 1.992 (4H, m), 1.758 (1H, m). ^{13}C-NMR ($CDCl_3$): δ = 170.07, 169.96, 70.02, 69.18, 67.07 64.83, 29.56, 20.98, 20.83.

Diacetate of 104 (458.2 mg, 2.266 mmol) was dissolved in anhydrous MeOH (5 mL) and the solution was cooled to 0ºC. Sodium methoxide (61.8 mg, 1.144 mmol) was added at 0ºC, and the mixture was stirred under an argon atmosphere for 2.5 h at room temperature. The solution was evaporated to remove the MeOH and the residue was chromatographed (AcOEt to CHCl3:MeOH = 9:1). For further purification, the residue was chromatographed (AcOEt to $CHCl_3$:MeOH = 9:1) to yield **104** (white solid, 247.5 mg, 2.095 mmol, 92%). ^{1}H-NMR ($CDCl_3$): δ = 4.037 (1H, d, *J* = 2.8 Hz), 3.913 (3H, m), 3.566-3.432 (2H, m), 3.382 (1H, dt, *J* = 2.3, 11.7 Hz), 3.090 (1H, dd, *J* = 9.6, 11.2 Hz), 1.944 (1H, m), 1.657-1.577 (1H, m). ^{13}C-NMR ($CDCl_3$): δ = 72.77, 71.86, 70.12, 66.22, 33.01.

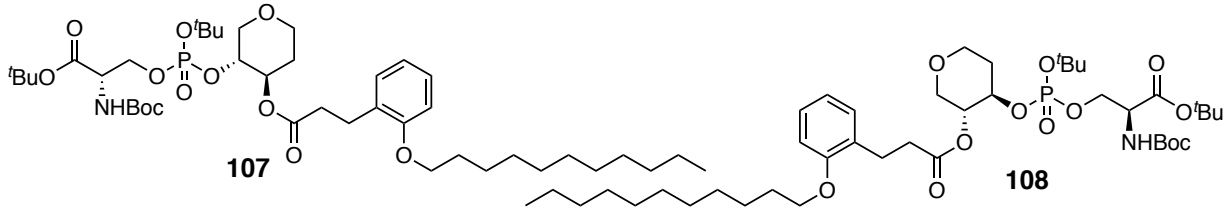
Synthesis of 105 and 106



The carboxylic acid **C3-ph-o-O-C11 (B)** (242.8 mg, 0.758 mmol), DMAP (23.1 mg, 0.189 mmol), and EDCI・HCl (223.9 mg, 1.168 mmol) were dissolved in anhydrous CH_2Cl_2 (6 mL). A solution of diol **104** (120.4 mg, 1.019 mmol) in anhydrous CH_2Cl_2 (5 mL) was added at 0ºC,

and the mixture was stirred under an argon atmosphere for 1 h at 0 to 15ºC and for 6.5 h at room temperature. The reaction was quenched with water (20 mL) and the mixture was extracted three times with CH_2Cl_2 (20 mL). The combined organic layer was washed with brine (20 mL). The combined aqueous layer was extracted with CH_2Cl_2 (20 mL). The whole organic solution was dried over Na_2SO_4 and filtered. The filtrate was evaporated and the residue was chromatographed (n-hexane:AcOEt = 4:1) to yield a mixture of **105** and **106** (261.8 mg, 0.622 mmol, 82%, colorless oil). This mixture was directly used in the next reaction without separation of the regioisomers. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_{25}H_{40}NaO_5^+$: 443.2768. Found: 443.2774.

Synthesis of 107 and 108

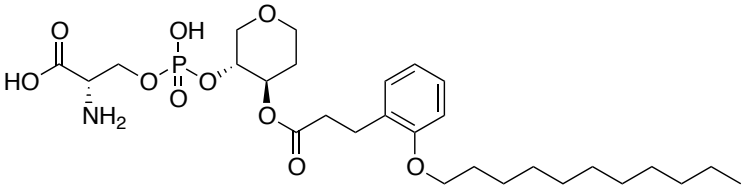


The mixture of **105** and **106** (261.8 mg, 0.622 mmol) was dissolved in CH_2Cl_2 and co-evaporated with toluene. **28** (433.4 mg, 0.933 mmol) was added, and the mixture was dissolved in CH_2Cl_2 and co-evaporated with toluene. Under an argon atmosphere, the residue was dissolved in anhydrous CH_2Cl_2 (5 mL), and a solution of 1*H*-tetrazole (88.9 mg, 1.269 mmol) in anhydrous THF (5 mL) was added at room temperature. After a few minutes, a white solid precipitated. The reaction mixture was stirred for 5 h at room temperature, then diluted with saturated $NaHCO_3$ aq. (15 ml) and extracted three times with CH_2Cl_2 (15 ml). The combined organic layer was washed with brine (10 mL). The combined aqueous layer was extracted with CH_2Cl_2 (15 mL). The whole organic solution was dried over Na_2SO_4 and filtered. The filtrate was evaporated and the residue was chromatographed (n-hexane: AcOEt: Et_3N = 35:4:1). The residue was dissolved in anhydrous CH_2Cl_2 (10 mL). TBHP in decane (5.0-6.0 M) (226 μL, 1.130 mmol) was added to the solution, and the mixture was stirred for 10.5 h at room temperature under an argon atmosphere. The solution was diluted with water (20 mL) and extracted three times with CH_2Cl_2 (20 mL). The combined organic layer was washed with brine (20 mL), dried over Na_2SO_4 and filtered. The filtrate was evaporated and the residue was chromatographed (n-hexane: AcOEt = 5:1 to 2:1) several times to yield **107** (27.6 mg, 0.035 mmol, 6%, colorless oil) and **108** (125.0 mg, 0.156 mmol, 25%, thick oil).

107: ^{1}H-NMR ($CDCl_3$): δ = 7.145 (2H, m), 6.828 (2H, m), 5.670 (1H, dd, *J* = 8.2, 16.2 Hz), 4.946 (1H, m), 4.348 (2H, m), 4.194 (2H, m), 3.926 (3H, m), 3.724 (1H, m), 3.517 (2H, m), 2.942 (2H, t, *J* = 7.4 Hz), 2.648 (2H, m), 2.084 (1H, m), 1.794 (2H, m), 1.552 (1H, m), 1.494-1.423 (29H, m), 1.394-1.265 (14H, m), 0.877 (3H, t, *J* = 6.8 Hz). ^{13}C-NMR ($CDCl_3$): δ = 172.36, 172.33, 172.28, 168.34, 168.30, 156.99, 156.95, 155.30, 129.94, 128.56, 128.53, 127.63, 127.59, 120.16, 111.04, 84.37, 84.30, 84.23, 84.16, 82.68, 82.57, 79.95, 79.90, 72.53, 72.48, 72.43, 72.41, 70.11, 70.03, 67.95, 67.84, 67.79, 67.49, 64.43, 64.22, 54.46, 54.38, 34.16, 34.11, 34.08, 31.89, 29.79, 29.76, 29.72, 29.60, 29.37, 29.32, 28.93, 28.31, 27.93, 26.14, 26.13, 22.66, 14.09. ^{31}P-NMR ($CDCl_3$): δ = -6.34. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_{36}H_{58}NNaO_9PSi^+$: 822.4528. Found: 822.4505.

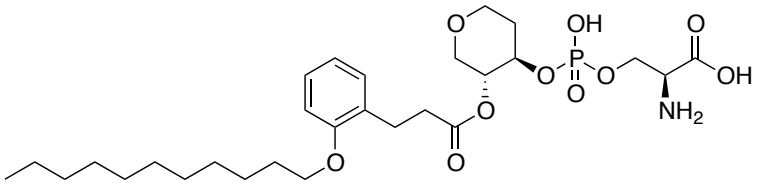
108: ^{1}H-NMR ($CDCl_3$): δ = 7.143 (2H, m), 6.826 (2H, m), 5.576 (1H, m), 4.800 (1H, m), 4.452-4.302 (3H, m), 4.202 (1H, m), 3.948 (2H, t, *J* = 6.4 Hz), 3.850 (2H, m), 3.526 (1H, m), 3.383 (1H, m), 2.940 (2H, m), 2.655 (2H, m), 2.129 (1H, m), 1.773 (3H, m), 1.538-1.439 (29H, m), 1.365-1.264 (14H, m), 0.875 (3H, t, *J* = 6.6 Hz). ^{13}C-NMR ($CDCl_3$): δ = 172.31, 172.28, 168.30, 156.92, 155.25, 129.94, 128.57, 128.53, 127.59, 127.55, 120.13, 111.00, 84.28, 84.21, 84.04, 83.97, 82.68, 82.60, 79.94, 73.13, 73.07, 72.68, 72.66, 72.60, 69.36, 69.28, 69.15, 69.08, 67.77, 67.63, 67.57, 66.41, 66.17, 63.94, 63.58, 54.42, 54.34, 34.04, 33.98, 31.87, 30.31, 29.81, 29.76, 29.71, 29.59, 29.58, 29.57, 29.35, 29.30, 29.29, 28.30, 27.92, 26.09, 26.03, 22.64, 14.07. ^{31}P-NMR ($CDCl_3$): δ = -6.33, -6.75. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_{36}H_{58}NNaO_9PSi^+$: 822.4528. Found: 822.4506.

Synthesis of 20



107 (27.4 mg, 0.034 mmol) was dissolved in 1,3-dimethoxybenzene (100 μL) and the solution was cooled to 0°C. TFA (0.5 mL) was added, and the mixture was stirred for 1 h at 0 to 10°C and for 11 h at 20 to 25°C, then diluted with CH_2Cl_2 (5 mL) and evaporated. The residue was chromatographed ($CHCl_3$:MeOH:AcOH = 9:1:0, 9:1:1, 8:1:1 to 7:1:2) to yield **20** as the AcOH salt (19.8 mg, 0.034 mmol, quant, white solid). The AcOH salt was dissolved in TFA and evaporated to yield **20** as the TFA salt (white solid). ^{1}H-NMR ($CDCl_3$): δ = 7.212 (1H, t, *J* = 7.6 Hz), 7.062 (1H, d, *J* = 6.8 Hz), 6.895 (2H, m), 5.033 (1H, brs), 4.665 (2H, brs), 4.545 (1H, brs), 4.194 (1H, brs), 4.032 (2H, t, *J* = 6.6 Hz), 3.802 (4H, m), 2.988 (2H, m), 2.815 (2H, t, *J* = 7.2 Hz), 2.179 (2H, m), 1.813 (2H, quintet, *J* = 7.0 Hz), 1.601 (1H, m), 1.455 (2H, m), 1.378-1.280 (14H, m), 0.878 (3H, t, *J* = 6.6 Hz). ^{31}P-NMR ($CDCl_3$/TFA-d): δ = -2.80. HRMS (ESI-TOF, [M-H]$^-$): Calcd for $C_{28}H_{45}NO_{10}P^-$: 586.2787. Found: 586.2786. Mp: 147.8°C-148.8°C, white cubes. Anal. Calcd. for $C_{28}H_{46}NO_{10}P \cdot 0.8CF_3COOH$: C, 52.37; H, 6.95; N, 2.06. Found: C, 52.15; H, 7.05; N, 2.00.

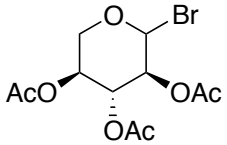
Synthesis of 23



108 (141.2 mg, 0.177 mmol) was dissolved in 1,3-dimethoxybenzene (0.5 mL) and the solution was cooled to 0°C. TFA (2 mL) was added, and the mixture was stirred for 11 h at 0 to 25°C, then diluted with CH_2Cl_2 (10 mL) and evaporated. The residue was chromatographed ($CHCl_3$:MeOH:AcOH = 9:1:0, 10:1:1, 9:1:1, 8:1:1 to 7:1:2) to yield **23** as the AcOH salt (92.1 mg, 0.157 mmol, 89%, colorless solid). The AcOH salt was dissolved in TFA and evaporated to yield **23** as the TFA salt (white solid). ^{1}H-NMR ($CDCl_3$/TFA-d): δ = 7.202 (1H, m), 7.053 (1H, d, *J* = 6.4 Hz), 6.879 (2H, m), 4.839 (1H, brs), 4.648 (2H, brs), 4.468 (2H, m), 3.962 (4H, m), 3.825 (1H, m), 3.714 (1H, dd, *J* = 2.8, 12.4 Hz), 2.954 (2H, m), 2.782 (2H, t, *J* = 7.2 Hz), 2.108 (1H, m), 1.791 (3H, m), 1.436 (2H, m), 1.363-1.228 (14H, m), 0.879 (3H, t, *J* = 6.8 Hz). ^{31}P-NMR ($CDCl_3$/TFA-d): δ = -2.77. HRMS (ESI-TOF, [M-H]$^-$): Calcd for $C_{28}H_{45}NO_{10}P^-$: 586.2787. Found: 586.2786. Mp: 122.0°C-123.0°C, colorless cubes. Anal. Calcd. for $C_{28}H_{46}NO_{10}P \cdot 0.5CF_3COOH$: C, 54.03; H, 7.27; N, 2.17. Found: C, 54.21; H, 7.34; N, 2.14.

Synthesis of cyclic 21 and 25 (Figure 38)

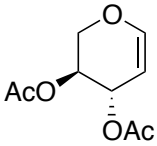
Synthesis of 109



L-Xylose (3.0042 g, 20.0117 mmol) was dissolved in pyridine (30 mL) and the solution was cooled to 0°C. Acetic anhydride (16.3852 g, 160.498 mmol) was added, and the reaction mixture was stirred for 7 h at room temperature under an argon atmosphere. Toluene was added, and the mixture was evaporated three times (total 80 mL). CH_2Cl_2 (150 mL) was added and the resulting mixture was extracted with 2 N HCl (40 mL) and 2 N NaOH (40 mL), then washed with brine

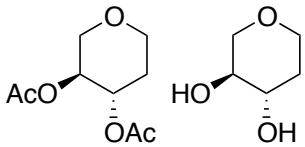
(50 mL). The combined aqueous layer was extracted three times with CH_2Cl_2 (20 mL). The combined organic layer was extracted with washed with brine (30 mL). The whole organic solution was dried over Na_2SO_4 and filtered. The filtrate was evaporated to give the tetraacetate (colorless oil). The crude product was directly used in the next step. A solution of HBr/AcOH (25 wt %, 13.0393 g, 40.289 mmmol) was added to the crude tetraacetate in anhydrous $CHCl_3$ (20 mL) at 0ºC. The mixture was stirred for 12.5 h at 0 to 10ºC, then CH_2Cl_2 (100 mL) was added. The resulting mixture was extracted with ice-water (100 mL), iced sodium bicarbonate solution (100 mL), and ice-water (100 mL), then washed with brine (100 mL). The organic layer was dried over $CaCl_2$, and filtered. The filtrate was evaporated. Et_2O was added to the residue and the mixture was kept at -25ºC. Filtration and washing with Et_2O gave **109** (brown solid, 5.0771 g, 14.971 mmol, 75 %, two steps). ^{1}H-NMR ($CDCl_3$): δ = 6.579 (1H, d, *J* = 4.0 Hz), 5.564 (1H, t, *J* = 9.6 Hz), 5.039 (1H, m), 4.772 (1H, dd, *J* = 4.0, 10.0 Hz), 4.050 (1H, dd, *J* = 5.8, 11.4 Hz), 3.880 (1H, t, *J* = 11.0 Hz), 2.098 (3H, s), 2.057 (3H, s), 2.055 (3H, s). ^{13}C-NMR ($CDCl_3$): δ = 169.83, 169.73, 87.57, 70.88, 69.54, 68.09, 62.53, 20.65, 20.64, 20.61. Mp: 65.5-66.5°C, gray solid.

Synthesis of 110



To a hot solution of cupric acetate monohydrate (644.8 mg, 5.389 mmol) in 20 mL of acetic acid was added zinc (11.1812 g, 171.019 mmol) at 90ºC. The oil bath was removed, the solution was stirred for 90 seconds at room temperature and the acetic acid was removed by evaporation. Acetic acid (4 mL) was added to the residue, and the mixture was stirred for 90 seconds, then the acetic acid was removed. This procedure was repeated another four times. Anhydrous Et_2O (5 mL) was added to the residue, and the mixture was stirred for 90 seconds, then the Et_2O was removed. This was repeated another two times to yield the zinc-copper couple. To the prepared zinc-copper couple, anhydrous THF (80 mL) and AcOH (4 mL) were added. AcONa (483.7 mg, 5.897 mmol) and **109** (1.9958 g, 5.885 mmol) were added at 0ºC, and the mixture was stirred for 1 h at the same temperature and for 11 h at room temperature. The reaction mixture was filtered through Celite®. The filtrate was washed with CH_2Cl_2 (200 mL), extracted twice with saturated $NaHCO_3$ (100 mL), washed with brine (100 mL), dried over Na_2SO_4, and filtered. The filtrate was evaporated and the residue was chromatographed (n-hexane:AcOEt=3:1) to yield **110** (colorless oil, 642.0 mg, 3.207 mmol, 54%). ^{1}H-NMR ($CDCl_3$): δ = 6.566 (1H, d, *J* = 5.6 Hz), 4.939 (3H, m), 4.161 (1H, m), 3.944 (1H, dd, *J* = 1.8, 12.2 Hz), 2.064 (3H, s), 2.033 (3H, s). ^{13}C-NMR ($CDCl_3$): δ = 169.81, 169.70, 147.94, 97.32, 67.12, 63.53, 63.34, 21.01, 20.82. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_9H_{12}NaO_5^+$: 223.0577. Found: 223.0595.

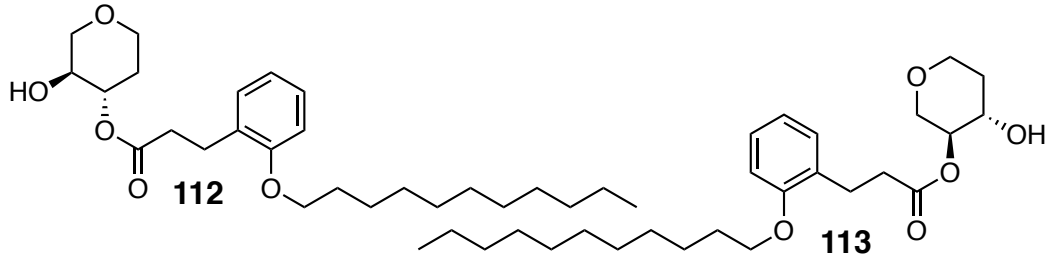
Synthesis of 111



110 (1.6317 g, 8.151 mmol) was dissolved in anhydrous MeOH (20 mL), and Pd-C (158.1 mg) in anhydrous methanol was added to the solution. Under an H_2 atmosphere, the mixture was stirred for 14.5 h, then filtered through Celite®. The filtrate was evaporated, and the residue was chromatographed (n-hexane:AcOEt = 3:1 to 2:1) to yield **diacetate of 111** (colorless oil, 731.1 mg, 3.616 mmol, 44%). ^{1}H-NMR ($CDCl_3$): δ = 4.938 (1H, dt, *J* = 4.5, 8.1 Hz), 4.809 (1H, dt, *J* = 4.0, 7.6 Hz), 3.959 (1H, dt, *J* = 4.0, 11.6 Hz), 3.849 (1H, dt, *J* = 4.7, 11.9 Hz), 3.515 (1H, m), 3.371 (1H, dd, *J* = 7.6, 11.9 Hz), 2.091 (7H, m), 1.704 (1H, m). ^{13}C-NMR ($CDCl_3$): δ = 170.09, 169.98, 70.03, 69.19, 67.08 64.84, 29.57, 21.00, 20.84. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_9H_{14}NaO_5^+$: 225.0733. Found: 225.0741.

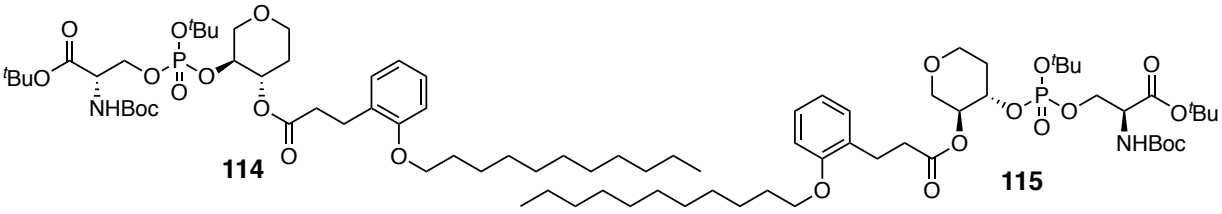
Diacetate of 111 (704.7 mg, 3.485 mmol) was dissolved in anhydrous MeOH (7 mL) and the solution was cooled to 0°C. Sodium methoxide (98.8 mg, 1.829 mmol) was added at 0°C and the reaction mixture was stirred under an argon atmosphere for 1.5 h at room temperature, then evaporated to remove the MeOH. The residue was chromatographed ($CHCl_3$:MeOH = 9:1) to yield **111** (white solid, 361.2 mg, 3.058 mmol, 88%). ^{1}H-NMR ($CDCl_3$): δ = 4.200 (1H, d, *J* = 4.0 Hz), 4.087 (1H, d, *J* = 3.2 Hz), 3.904 (2H, m), 3.561-3.423 (2H, m), 3.377 (1H, dt, *J* = 2.3, 11.8 Hz), 3.087 (1H, dd, *J* = 9.6, 10.8 Hz), 1.938 (1H, m), 1.651-1.551 (1H, m). ^{13}C-NMR ($CDCl_3$): δ = 72.74, 71.82, 70.12, 66.22, 33.02. Mp: 64.0-65.6°C, gray solid.

Synthesis of 112 and 113



The diol **111** (123.2 mg, 1.043 mmol), DMAP (21.0 mg, 0.172 mmol), and EDCI・HCl (214.2 mg, 1.117 mmol) were dissolved in CH_2Cl_2 (10 mL), and a solution of carboxylic acid **C3-ph-o-O-C11 (B)** (271.1 mg, 0.846 mmol) and CH_2Cl_2 (2 mL) were added. The mixture was stirred for 8 h at room temperature under an argon atmosphere. The reaction was quenched with water (20 mL) and the mixture was extracted three times with CH_2Cl_2 (20 mL). The combined organic layer was washed with brine (20 mL). The combined aqueous layer was extracted with CH_2Cl_2 (20 mL). The combined organic layer was dried over Na_2SO_4 and filtered. The filtrate was evaporated and the residue was chromatographed (n-hexane:AcOEt = 4:1 to 3:1) to yield a mixture of **112** and **113** (269.2 mg, 0.640 mmol, 76%, colorless oil). This mixture was directly used in the next reaction without separation of the regioisomers. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_{25}H_{40}NaO_5^+$: 443.2768. Found: 443.2762.

Synthesis of 114 and 115



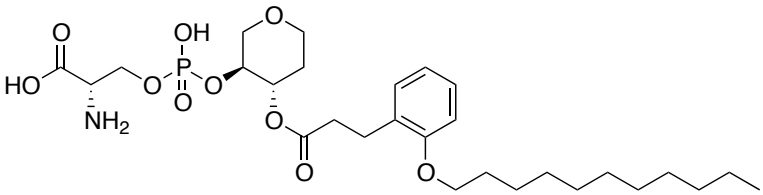
The mixture of **112** and **113** (266.1 mg, 0.538 mmol) was dissolved in CH_2Cl_2 and co-evaporated with toluene. **28** (443.3 mg, 0.954 mmol) was added. The mixture was dissolved in CH_2Cl_2 and co-evaporated with toluene. Under an argon atmosphere, the residue was dissolved in anhydrous CH_2Cl_2 (5 mL), and a solution of 1*H*-tetrazole (90.8 mg, 1.296 mmol) in anhydrous THF (4.8 mL) was added at room temperature. After a few minutes, a white solid precipitated. The reaction mixture was stirred for 5.5 h at room temperature, then diluted with saturated $NaHCO_3$ aq. (25 mL). The resulting mixture was extracted three times with CH_2Cl_2 (20 mL). The combined organic layer was washed with brine (20 mL). The combined aqueous layer was extracted with CH_2Cl_2 (20 mL). The whole organic solution was dried over Na_2SO_4 and filtered. The filtrate was evaporated and the residue was chromatographed (n-hexane:AcOEt:Et_3N = 35:4:1). The residue was dissolved in anhydrous CH_2Cl_2 (10 mL), and TBHP in decane (5.0-6.0 M) (226 μL, 1.130 mmol) was added. The mixture was stirred for 11.5 h at room temperature under an argon atmosphere. The solution was diluted with water (20 mL) and extracted three times with CH_2Cl_2 (20 mL). The combined organic layer was washed with brine (20 mL). The combined aqueous layer was extracted with CH_2Cl_2 (20 mL). The combined organic layer was dried over Na_2SO_4 and filtered. The filtrate was evaporated and the residue was chromatographed (n-hexane:AcOEt

= 4:1 to 2:1) several times to yield **114** (77.7 mg, 0.097 mmol, 18%, colorless oil) and **115** (116.1 mg, 0.145 mmol, 27%, thick oil).

114: ^{1}H-NMR ($CDCl_3$): δ = 7.138 (2H, m), 6.818 (2H, m), 5.533 (1H, m), 4.938 (1H, m), 4.320 (2H, m), 4.202 (2H, m), 3.930 (3H, m), 3.724 (1H, m), 3.476 (2H, m), 2.935 (2H, m), 2.656 (2H, m), 2.074 (1H, m), 1.786 (2H, quintet, *J* = 7.0 Hz), 1.642-1.509 (1H, m), 1.445 (29H, m), 1.381-1.257 (14H, m), 0.868 (3H, t, *J* = 6.8 Hz). ^{13}C-NMR ($CDCl_3$): δ = 172.29, 168.27, 168.23, 156.91, 155.21, 129.90, 128.53, 128.51, 127.57, 127.54, 120.12, 111.00, 84.33, 84.29, 84.22, 84.17, 84.11, 84.04, 82.61, 79.83, 79.81, 72.69, 72.63, 72.54, 72.48, 70.25, 70.18, 70.08, 70.06, 70.02, 69.99, 67.85, 67.74, 67.64, 67.55, 66.49, 64.49, 64.40, 54.49, 54.41, 54.33, 34.12, 34.05, 31.84, 29.73, 29.68, 29.56, 29.32, 29.28, 29.06, 28.89, 28.26, 27.90, 26.09, 22.61, 14.04. ^{31}P-NMR ($CDCl_3$): δ = -6.37, -6.43. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_{36}H_{58}NNaO_9PSi^+$: 822.4528. Found: 822.4515.

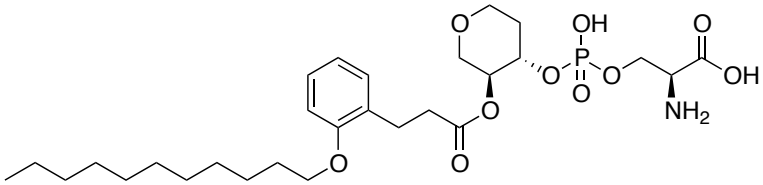
115: ^{1}H-NMR ($CDCl_3$): δ = 7.126 (2H, m), 6.809 (2H, m), 5.523 (1H, m), 4.786 (1H, m), 4.448-4.286 (1H, m), 4.203 (1H, m), 3.931 (2H, t, *J* = 6.6 Hz), 3.897-3.763 (2H, m), 3.502 (1H, m), 3.350 (1H, m), 2.931 (2H, m), 2.639 (2H, m), 2.116 (1H, m), 1.761 (3H, m), 1.502-1.399 (29H, m), 1.373-1.249 (14H, m), 0.859 (3H, t, *J* = 7.0 Hz). ^{13}C-NMR ($CDCl_3$): δ = 172.18, 168.21, 168.15, 156.83, 155.11, 129.85, 128.46, 128.43, 127.49, 127.47, 120.05, 110.91, 84.13, 84.06, 83.97, 83.89, 82.54, 79.82, 79.78, 73.17, 73.13, 72.96, 72.90, 69.33, 69.25, 69.11, 69.04, 67.67, 67.46, 67.40, 66.37, 66.15, 63.95, 63.62, 54.43, 54.33, 54.25, 53.30, 33.95, 33.89, 31.78, 30.38, 29.87, 29.69, 29.67, 29.65, 29.63, 29.50, 29.49, 29.47, 29.26, 29.21, 28.19, 27.84, 27.82, 26.00, 22.55, 13.98. ^{31}P-NMR ($CDCl_3$): δ = -6.26, -6.61. HRMS (ESI-TOF, $[M+Na]^+$): Calcd. for $C_{36}H_{58}NNaO_9PSi^+$: 822.4528. Found: 822.4537.

Synthesis of 21



114 (77.4 mg, 0.097 mmol) was dissolved in 1,3-dimethoxybenzene (0.5 mL) and the solution was cooled to 0°C. TFA (2 mL) was added, and the mixture was stirred for 1 h at 0°C and for 11 h at 20 to 25^{0}C, then diluted with CH_2Cl_2 (10 mL). The resulting mixture was evaporated, and the residue was chromatographed ($CHCl_3$:MeOH:AcOH = 9:1:0, 10:1:1, 9:1:1, 8:1:1 to 7:1:2) to yield **22** as the AcOH salt (58.3 mg, 0.099 mmol, quant, white solid). The AcOH salt was dissolved in TFA and evaporated to yield **22** as the TFA salt (white solid). ^{1}H-NMR ($CDCl_3$/TFA-d): δ = 7.200 (1H, m), 7.077 (1H, m), 6.869 (2H, m), 5.048 (1H, brs), 4.676 (2H, brs), 4.498 (1H, brs), 4.285 (1H, brs), 4.007 (2H, t, *J* = 6.6 Hz), 3.750 (4H, m), 2.971 (2H, m), 2.787 (2H, t, *J* = 6.8 Hz), 2.195 (1H, brs), 1.803 (2H, m), 1.609 (1H, d, *J* = 12.8 Hz), 1.455 (2H, m), 1.375-1.277 (14H, m), 0.881 (3H, t, *J* = 6.8 Hz). ^{31}P-NMR ($CDCl_3$/TFA-d): δ = -2.80. HRMS (ESI-TOF, $[M-H]^-$): Calcd for $C_{28}H_{45}NO_{10}P^-$: 586.2787. Found: 586.2835. Mp: 96.5°C-98.5°C, white cubes. Anal. Calcd. for $C_{28}H_{46}NO_{10}P \cdot 0.8CF_3COOH$: C, 52.37; H, 6.95; N, 2.06. Found: C, 52.22; H, 6.97; N, 1.98.

Synthesis of 25



115 (115.4 mg, 0.144 mmol) was dissolved in 1,3-dimethoxybenzene (0.5 mL) and the solution was cooled to 0°C. TFA (2 mL) was added, and the mixture was stirred for 10 h at 0 ^{0}C to room temperature, then diluted with CH_2Cl_2 (10 mL) and evaporated. The residue was chromatographed ($CHCl_3$/MeOH/AcOH = 10:1:1, 9:1:1, 8:1:1 to 7:1:2) twice to yield **25** as the AcOH salt (81.4 mg, 0.139 mmol, 97%, white solid). The AcOH salt was dissolved in TFA and evaporated to yield

25 as the TFA salt (white solid). ^{1}H-NMR ($CDCl_3$/TFA-d): δ = 7.200 (1H, t, *J* = 7.4 Hz), 7.045 (1H, d, *J* = 7.2 Hz), 6.868 (2H, m), 4.817 (1H, brs), 4.625 (2H, brs), 4.478 (2H, brs), 4.013-3.809 (5H, m), 3.712 (1H, d, *J* = 11.2 Hz), 2.945 (2H, m), 2.772 (2H, t, *J* = 6.8 Hz), 2.076 (1H, brs), 1.793 (3H, m), 1.442 (2H, m), 1.367-1.281 (14H, m), 0.883 (3H, t, *J* = 6.6 Hz). ^{31}P-NMR ($CDCl_3$/TFA-d): δ = -3.05. HRMS (ESI-TOF, $[M-H]^-$): Calcd for $C_{28}H_{45}NO_{10}P^-$: 586.2787. Found: 586.2827. Mp: 132.8ºC-134.8ºC, white cubes. Anal. Calcd. for $C_{28}H_{46}NO_{10}P \cdot 0.5CF_3COOH$: C, 54.03; H, 7.27; N, 2.17. Found: C, 53.68; H, 7.24; N, 2.10.

Biological Evaluations

TGFα shedding assay

For P2Y10 and zebrafish GPR34, transfection was carried out as follows: HEK293A cells were seeded in 100 mm dishes at a density of 2×10^6 cells per dish and, after incubation for 24 h, transfected with a mixture of plasmids (alkaline phosphatase-tagged TGFα (AP-TGFα, 2.5 μg), and mouse P2Y10 (2.5 μg), zebrafish GPR34 type a (1 μg) or zebrafish GPR34 type b (1 μg)) in 1 mL of Polyethyleneimine Max Solution (20 μg/mL). For zebrafish GPR34 type a and b, a plasmid encoding chimeric $G\alpha_{q/i1}$ subunit (0.5 μg) was co-transfected. Evaluation of GPCRs activation and the AP assay (TGFα shedding assay) were performed as described previously.[14]

Ca^{2+} mobilization assay

Ca^{2+} mobilization assay was performed as described previously.[7] Briefly, HEK293A cells were transfected with mouse GPR34 and $G\alpha_{q/i1}$-expressing plasmids and loaded with a fluorescent Ca^{2+} indicator, FLIPR Calcium 5 Assay Kit, and the intracellular Ca^{2+} concentration was determined by measuring the fluorescence intensity.

Computational Simulations

Monitoring dihedral angles of LysoPS analogues in REST simulations

The selected dihedral angles (**P**, **FA**, see Figure 11) were calculated during REST simulations,[20] in which the analogue was placed either in water or n-octanol. The simulations were performed by Desmond (version 3.8.5.19, Schrödinger 2018-1, Schrödinger, LLC, NY)[23] using the OPLS3 force field.[24]

The structures of the ligands were prepared by using LigPrep (version 45011, Schrödinger 2018-1, Schrödinger, LLC, NY) and the output with total charge -1 was used. The ligand was solvated with a 10 Å width buffer of TIP3P water with 0.15 M NaCl or n-octanol. Then, after equilibration of the system, the REST simulation including 4 replicas was performed. The production MD was performed in NPT ensemble at 300 K and 1.01325 bar. The effective temperature of the solute ligand ranges from 300 to 446 K (**1a** and **14**), 300 to 443 K (**2*R***, **2*S***, **3*R*** and **3*S***) or 300 to 437 K (**18**-**25**), determined by setting the acceptance ratio to 0.3. Each replica was run for 30 ns.

Generation of a GPR34 binding model complexed with compound 14 and REST simulation

We docked the high-affinity GPR34 agonist **14**, prepared by using LigPrep, to the GPR34 homology model we previously reported[14] by using Glide SP mode (version 78011,

Schrödinger 2018-1, Schrödinger, LLC, NY).[25, 26] The ligand structure having total charge –1 was used for docking. The homology model used for docking contains bound agonist **REF** (Figure 18 and Figure S5) and we defined the centroid of **REF** to be the grid center. Assuming that the benzene-containing fatty acid part of compound **14** takes the same binding position as that of the bound **REF** compound in our previously reported binding model, we constrained the docking position of compound **14** to the reference position, including the tri-benzene fatty acid moiety (see Figure 18 and Figure S5).

The resulting complex was placed in 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE) membrane and solvated with TIP3P water and 0.15 M NaCl. REST simulation, which included 4 replicas, was performed by using Desmond. The production MD was performed in NPγT ensemble at 300 K and 1.01325 bar with a surface tension of 4000 bar•Å. The effective temperature of the solute ligand ranges from 300 to 446 K, determined by setting the acceptance ratio to 0.3, and each replica was run for 30 ns. The final frame of the REST simulation was drawn in Figure 14 using the graphical user interface of Maestro (Schrödinger 2019-3, Schrödinger, LLC, NY).

ASSOCIATED CONTENT

Supporting Information Available:

Supporting Figures S1-S9

Molecular formula strings

A PDB file of docking model

These materials are available free of charge via the internet at http://pubs.asc.org.

AUTHOR INFORMATION

S. N. M. S., J. S., M. I. Y. O. and T. O. (University of Tokyo) performed the chemical studies including design and synthesis of compounds and simulations. A. U., K. K., J. O., A. I. and J. A. (Tohoku University and University of Tokyo) performed biological studies including TGFα shedding assay and Ca^{2+} mobilization assay. S. N. (design and synthesis of molecules), M. S. (simulations) and A. U. (biological assay) contributed equally to this work.

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NOTES

The authors declare no competing financial interests.

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