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Conformational Constraint of the Glycerol Moiety of Lysophosphatidylserine Affords Compounds with Receptor Subtype Selectivity

Sejin Jung,[†,§] Asuka Inoue,[‡,∥] Sho Nakamura,[†] Takayuki Kishi,[‡] Akiharu Uwamizu,[‡] Misa Sayama,[†] Masaya Ikubo,[†] Yuko Otani,[†] Kuniyuki Kano,[‡] Kumiko Makide,[‡,∥] Junken Aoki,*[,‡,⊥] and Tomohiko Ohwada*[,†]

[†]Laboratory of Organic and Medicinal Chemistry, Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

[‡]Laboratory of Molecular and Cellular Biochemistry, Graduate School of Pharmaceutical Sciences, Tohoku University, 6-3, Aoba, Aramaki, Aoba-ku, Sendai, Miyagi 980-8578, Japan

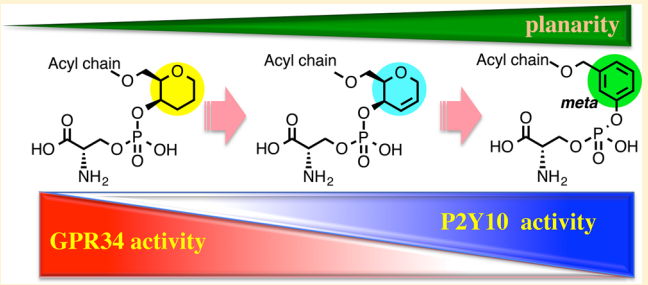
[§]Research Foundation ITSUU Laboratory, C1232 Kanagawa Science Park R & D Building, 3-2-1 Sakado, Takatsu-ku, Kawasaki, Kanagawa 213-0012, Japan

[∥]PRESTO, Japan Science and Technology Agency (JST), 4-1-8, Honcho, Kawaguchi-shi, Saitama 332-0012, Japan

[⊥]AMED-CREST, Japan Agency for Medical Research and Development, 1-7-1 Otemachi, Chiyoda-ku, Tokyo 100-0004, Japan

Supporting Information

ABSTRACT: Lysophosphatidylserine (LysoPS) is an endogenous lipid mediator that specifically activates membrane proteins of the P2Y and its related families of G protein-coupled receptors (GPCR), GPR34 (LPS_1), P2Y10 (LPS_2), and GPR174 (LPS_3). Here, in order to increase potency and receptor selectivity, we designed and synthesized LysoPS analogues containing the conformational constraints of the glycerol moiety. These reduced structural flexibility by fixation of the glycerol framework of LysoPS using a 2-hydroxymethyl-3-hydroxytetrahydropyran skeleton, and related structures identified compounds which exhibited high potency and selectivity for activation of GPR34 or P2Y10. Morphing of the structural shape of the 2-hydroxymethyl-3-hydroxytetrahydropyran skeleton into a planar benzene ring enhanced the P2Y10 activation potentcy rather than the GPR34 activation.



INTRODUCTION

Lysophosphatidylserine (LysoPS), which is derived from phosphatidylserine by enzymatic deacylation,[1] has lipid mediator-like actions, and induces multiple cellular responses both *in vitro* and *in vivo*, including mast cell degranulation,[2−5] neurite outgrowth in PC12 cells,[6] suppression of proliferation of T lymphocytes,[7] migration of fibroblasts and tumor cells,[8,9] and engulfment of apoptotic cells by macrophages.[10]

Recent studies to find ligands of orphan G-protein-coupled receptors (GPCRs) identified three LysoPS-specific receptors, namely P2Y10 (also known as LPS_2), A630033H20 (LPS_{2L}, (LPS_2-like)), and GPR174 (LPS_3) in addition to the previously identified LysoPS receptor (GPR34 (LPS_1)).[11−13] Orthologs of GPR34, P2Y10, and GPR174 have been found in human, mouse, rat, and zebrafish, and they respond to endogenous LysoPS with EC_{50} values at the submicromolar level.[14] The human *A630033H20* gene apparently encodes frame-shift product and is regarded as a a nonfunctional pseudogene. Therefore, there appear to be three LysoPS receptors of pharmaceutical significance. These receptors are mainly expressed in immune-related tissues,[15,16] and GPR174 was reported to be abundantly expressed in regulatory T cells.[17] Thus, it is plausible that LysoPS is involved in the pathogenesis of the immunity system. In order to provide useful tools for elucidation of the physiological roles of LysoPS and its specific receptors, we have conducted structure−activity relationship (SAR) studies by means of optimization of the individual structural modules of LysoPS (fatty acid, glycerol, and amino acid), as well as their linkages (Figure 1). Our results support the idea that LysoPS is assembled from modular components that influence subtype selectivity.[18,19]

Its modular structure and many rotatable single bonds make LysoPS very flexible, and it may adopt various active conformations that could account for its pan-agonistic activity toward GPR34, P2Y10, and GPR174. This idea led us to

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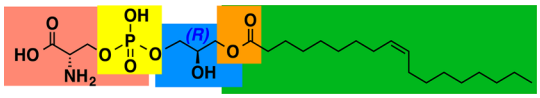

Figure 1. Structure of LysoPS (1-oleoyl-lysophosphatidylserine), showing its structural modules and linkers in different colors.

hypothesize that suitable conformational constraints could generate structures with selective receptor-activating activity. Therefore, in this study, we designed and synthesized a number of conformationally constrained LysoPS analogues by embedding the glycerol moiety into 2-hydroxymethyl-3-hydroxytetrahydropyran and related skeletons (see Figure 2), and we examined the selective receptor-activating ability of the resulting derivatives.

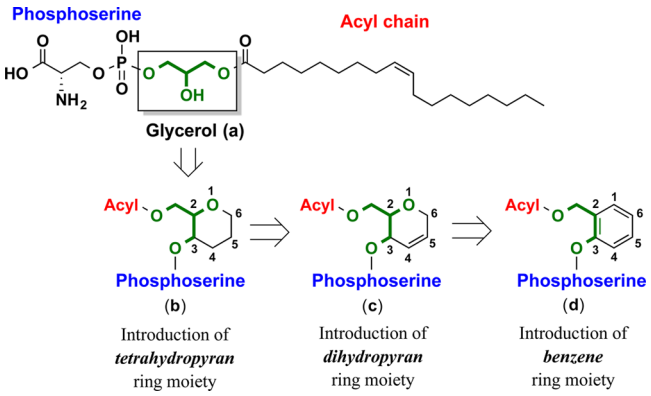

Figure 2. Generation of LysoPS analogues by replacing the glycerol module with various conformationally restricted structures.

RESULTS AND DISCUSSIONS

Molecular Design of Conformationally Constrained LysoPS Derivatives. LysoPS is composed of several substructural modules (amino acid, glycerol, and fatty acid chain) connected via linkers (phosphodiester and ester linkages), as shown in Figure 1. Our previous SAR studies indicated that L-serine is an essential module for the activities toward all the three LysoPS receptors, and LysoPS derivatives containing aromatized nonlipid surrogates of fatty acids are effective for activation of our target receptors.[18,19]

In addition, the hydroxyl group at the *sn*-2 position of the glycerol backbone has been proposed to have a key role in activation of GPR174, probably by acting as a hydrogen-bonding donor to the receptor.[18,19] Therefore, we introduced a 2-hydroxymethyl-3-hydroxytetrahydropyran skeleton (b) in place of the glycerol moiety (a) with the aim of increasing the receptor selectivity by deleting the hydrogen atom of the *sn*-2 hydroxyl group of glycerol (Figure 2). The introduced 2-hydroxymethyl-3-hydroxytetrahydropyran moiety (b, Figure 2) completely reproduces the three carbon and three oxygen atoms of the glycerol backbone (a, Figure 2).

In addition, its cyclic structure allows LysoPS analogues to generate two different regioisomers due to the nonequivalent substituents, i.e., one containing the acyl chain at the primary alcohol position, and the other containing the acyl chain at the secondary alcohol position (Figure 3). Thus, introduction of the tetrahydropyran moiety serves to restrict the ring conformation as well as the space-filling of the phosphodiester and acyl units. Advantages of 2-hydroxymethyl-3-hydroxytetrahydropyrans (b) include readily availability from commercially available sugar compounds and their potential for derivatization due to the nonequivalent primary and secondary alcohols and two stereogenic centers at the ring junction.

We also focused on 2-hydroxymethyl-3,6-dihydro-2*H*-pyran-3-ol (c, Figure 2) as an unsaturated cyclic LysoPS analogue. This unsaturated ring moiety exists in a half-chair conformation with the oxygen atom (**O1** in Figure 2) on one side and C2 on the other side of the plane formed by the remaining four atoms.[20,21] These conformational features may provide a more restricted glycerol skeleton by removing the 1,3-diaxial interaction, potentially leading to more stable structures and limitations on conformational flexibility with respect to the ring structure. Finally, we tried replacing this unsaturated ring (c) with a benzene ring (d, Figure 2), because the completely planar structure and limited conformational flexibility might generate a limited range of conformations with potential for increased selectivity and potency of ligands toward specific receptors.

The detailed molecular design is shown in Figure 3. The conformation of the glycerol moiety can be fixed in two ways,

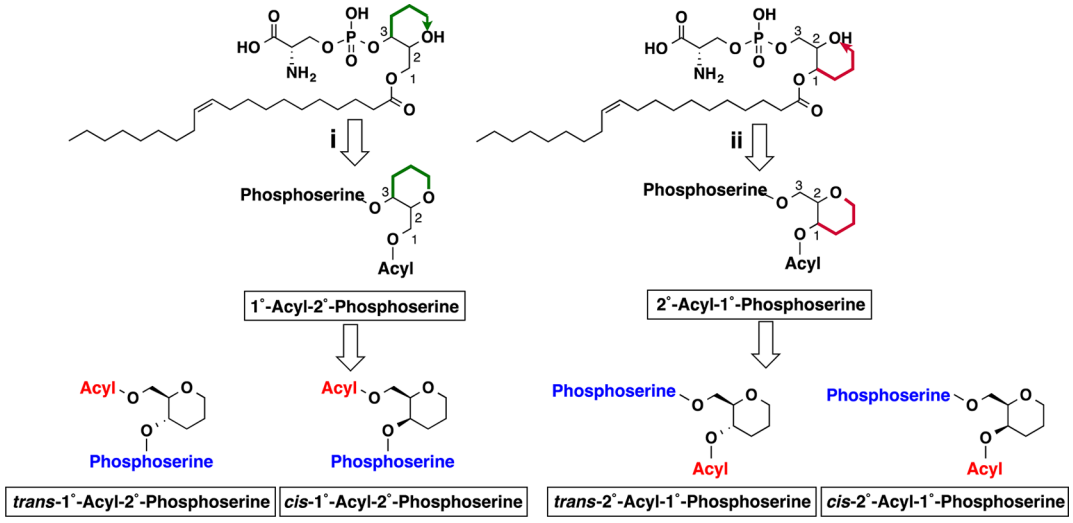

Figure 3. Design of cyclic LysoPS analogues by constraint of the glycerol moiety.

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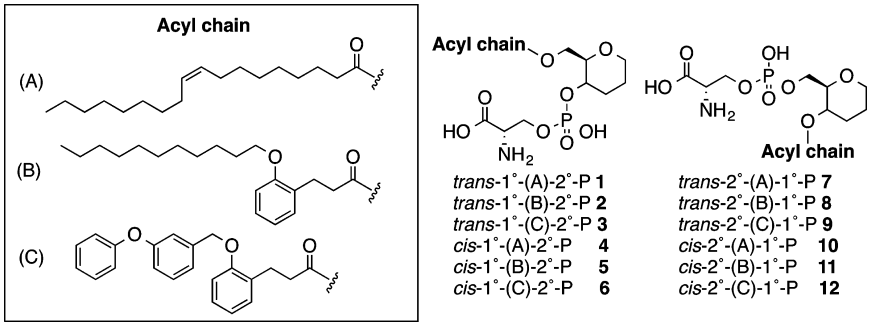

Figure 4. Saturated cyclic LysoPS analogues.

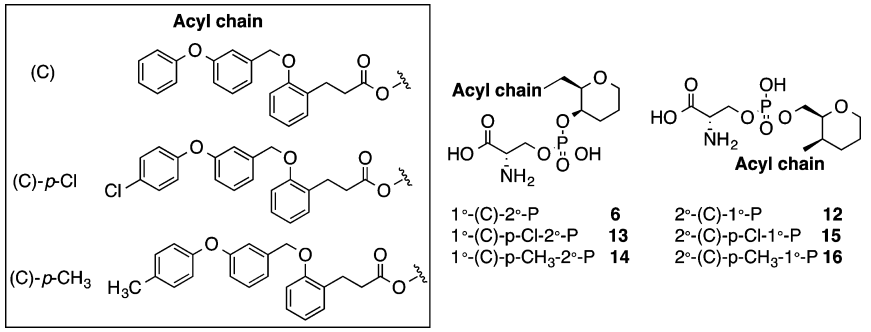

Figure 5. Modification of acyl chain (C): introducing substituents on the terminal benzene ring.

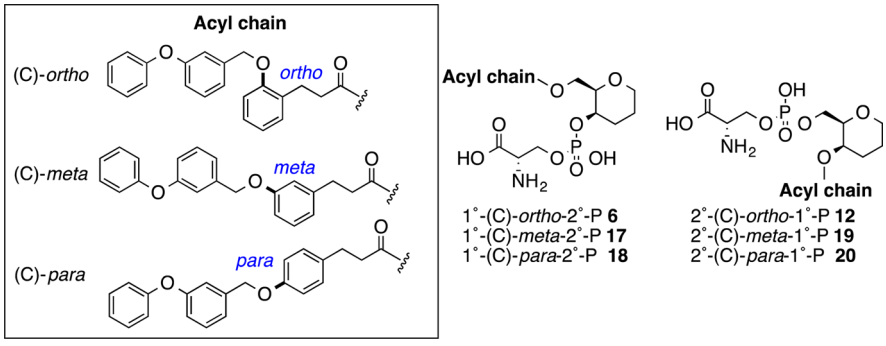

Figure 6. Investigation of isomers of acyl chain (C).

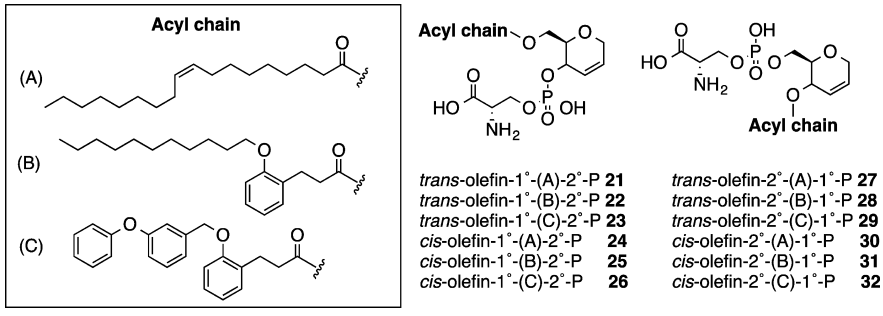

Figure 7. Unsaturated cyclic LysoPS analogues.

i.e., by linking carbon 3 to the hydroxyl group (**i**) or by linking carbon 1 to the hydroxyl group (**ii**). The former method generates 1°-acyl-2°-phosphoserine analogues and the latter generates 2°-acyl-1°-phosphoserine analogues (Figure 3). Moreover, the stereochemistry at the ring junction can take *trans* and *cis* configurations and provides four different frameworks of cyclic LysoPS analogues (*trans* and *cis*-1°-acyl-2°-phosphoserine; *trans* and *cis*-2°-acyl-1°-phosphoserine) (Figure 3).

Synthesis of LysoPS Derivatives Based on the 2-Hydroxymethyl-3-hydroxytetrahydropyran and Related Scaffolds. LysoPS analogues (**1**−**12**) based on the 2-hydroxymethyl-3-hydroxytetrahydropyran scaffold with all possible combinations arising from the presence of primary and secondary alcohols and *cis* and *trans* configurations were

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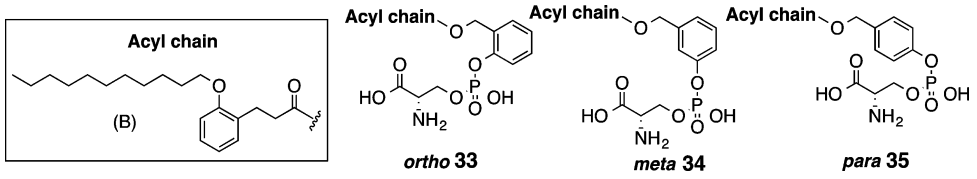
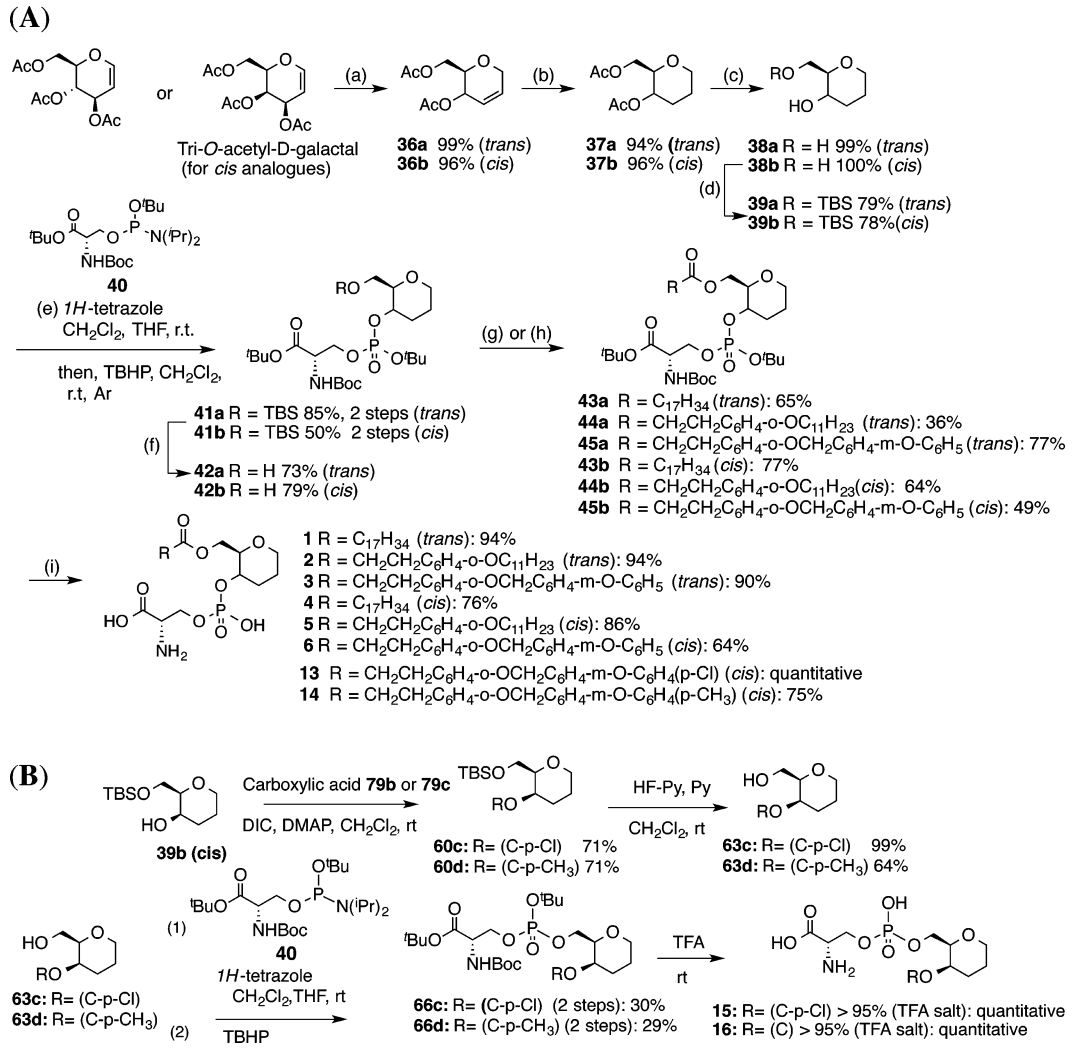


Figure 8. LysoPS analogues containing the aromatized glycerol moiety.

Scheme 1. Synthesis of 1°-Acyl-2°-phosphoserine-type Analogues (A) and 2°-Acyl-1°-phosphoserine-type Analogue (B)[a]



[a]Conditions for part A: (a) $HSiEt_3$, $BF_3{\cdot}OEt_2$, CH_2Cl_2, r.t. (b) Pd/C, MeOH. (c) NaOMe, MeOH, r.t. (d) TBSCl, imidazole, CH_2Cl_2, r.t. (f) Amberlyst15, MeOH, r.t., 99%. (g) Oleoyl chloride, DMAP, CH_2Cl_2, r.t. (h) R-CO_2H, diisopropylcarbodiimide, *N,N*-dimethylaminopyridine, CH_2Cl_2, r.t. (i) TFA, r.t.

designed (Figure 4). In this paper, we focused on three kinds of acyl chains, i. e., oleic acid (A), and its surrogates (B) and (C) (Figure 4), which were previously identified as potentially effective to activate specific receptors.[17,18] We also explored the subsituent effect of the acyl chain (C) (**13**−**16**, Figure 5) and the effects of the aromatic arrangemets toward GPR34 (**17**−**20**, Figure 6).

We also designed LysoPS anlogues (**21**−**32**) containing the unsaturated 2-hydroxymethyl-3,6-dihydro-2*H*-pyran-3-ol scaffold (Figure 7). Finally aromatized glycerol mimics (**33**−**35**) were designed (Figure 8). The synthesis of active derivatives are shown in the Experimental Section, and the synthesis of weak or no-active derivatives are shown in the Supporting Information.

Synthesis of Designed Molecules. The 2-hydroxymethyl-3-hydroxytetrahydropyran moiety was obtained through established synthetic methods from sugar compounds with appropriate stereochemistry (Scheme 1).[22,23] Tri-*O*-acetyl-D-glucal[22] was used as a starting material for trans derivatives, and tri-*O*-acetyl-D-galactal[23] was used for *cis* derivatives (Scheme 1). The starting sugars (tri-*O*-acetyl-D-glucal or tri-*O*-acetyl-D-galactal) were subjected to ionic hydrogenation ($HSiEt_3$ and $BF_3{\cdot}OEt_2$), followed by hydrogenation over Pd/C, and then hydrolysis of two acetyl groups with NaOMe/MeOH (i.e., via **36** and **37**) to obtain *trans*- and *cis*-2-hydroxymethyl-3-

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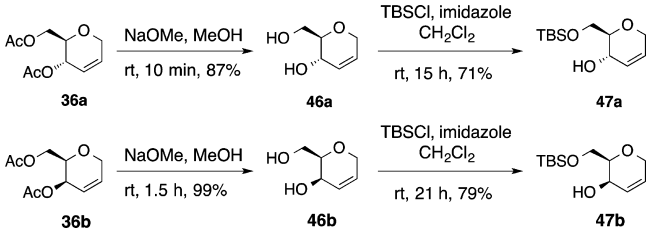
hydroxytetrahydropyrans (**38a** and **38b**, respectively). The primary alcohol was selectively protected with a TBS group (**39a** and **39b**), followed by formation of a phosphate diester linkage using the phosphoramidite method with compound **40**. Acid-catalyzed deprotection of the silyl ether (**41**) was carried out to free the primary alcohol **42a** (*trans*) or **42b** (*cis*), followed by acylation with various fatty acid analogues, and then complete deprotection of **43**–**45** in TFA furnished the desired LysoPS analogues, *trans*-1°-acyl-2°-phosphoserines (**1**–**3**) and *cis*-1°-acyl-2°-phosphoserines (**4**–**6**) (Scheme 1A). 2°-Acyl-1°-phosphoserine compounds (*trans*-(**7**–**9**) and *cis*-(**10**–**12**)) were also obtained simply by changing the reaction order of esterification and connection of the phosphoserine group through the intermediates (**58**–**66**, Scheme S1). The analogues containing a para-substituted benzene ring with an acyl chain (C) (**13**–**16**, Figure 5, Scheme 1B and Scheme S2) and the analogues containing a difiifferent arrangement of three aromatic rings of the acyl chain (C) (**17**–**20**, Figure 6) were also synthesized. The syntheses of the derivatives **17**–**20** were shown in the Supporting Information (Schemes S3 and S4).

A 3,6-dihydro-2*H*-pyran moiety was obtained as an intermediate by skipping the hydrogenation step to obtain unsaturated cyclic moiety **47a** or **47b** (Scheme 2).

Scheme 2. Synthesis of Modified Unsaturated Cyclic Glycerol Framework



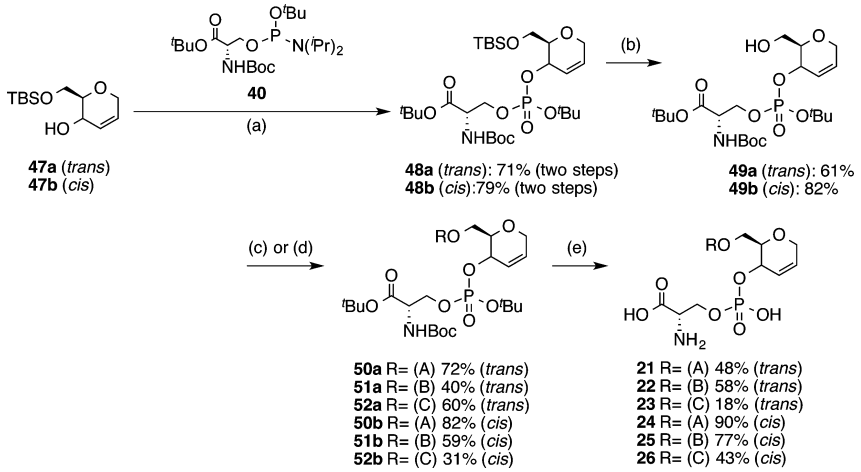
47a and **47b** were connected to phosphoserine by means of the phosphoramidite method (to give **48**) followed by deprotection of silylether to afford the free primary alcohol (**49**) and then esterification (**50**–**52**, Scheme 3). Finally, deprotection was carried out with TFA to afford the unsaturated primary acyl compounds (**21**–**26**). To obtain secondary acyl compounds (**27**–**32**, Figure 7), the order of connection of the phosphoserine unit and the ester was reversed through the intermediates (**67**–**75**, Scheme S5).

Three kinds of benzene-type LysoPS analogues, *ortho* (**33**), *meta* (**34**) and *para* (**35**), can be generated (Figure 6). To obtain these benzene-type LysoPS analogues, salicylic alcohol (*o*-hydroxybenzyl alcohol **53a**, *m*-hydroxybenzyl alcohol **53b** and *p*-hydroxybenzyl alcohol **53c**) were used (Scheme 4). The synthesis was performed as shown in Scheme 4: the benzyl alcohol was protected with a TBS group (**54**) and the phosphate group was connected to the phenolic oxygen atom (**55**), followed by deprotection of the TBS silylether (**56**), and then esterification of the benzyl alcohol to afford the protected benzene analogues (**57**). Deprotection in TFA gave the aromatic LysoPS derivatives (**33**-**35**).

Effect of Replacement of the Glycerol Backbone with a Tetrahydropyran Ring Moiety and Influence of Regioisomers. The agonistic activities of the synthesized cyclic LysoPS analogues toward GPR34 and P2Y10 in the TGFα shedding assay are summarized in Table 1 (see also Figure S1). The original concentration–response data are shown in Figure S2 (GPR34) and Figure S3 (P2Y10). All the LysoPS analogues synthesized and tested in this study lack the *sn*-2 hydroxy, which was found to be essential for the activation of GPR174. In fact, all the LysoPS analogues did not show significant activity toward GPR174 (*vide infra*).

Activity toward GPR34 1°-Acyl-2°-phosphoserine analogues (**1**–**6** except **6**) (**1**: EC_{50}= 100 nM; **2**: EC_{50}= 640 nM; **3**: EC_{50}= 340 nM; **4**: EC_{50}= 210 nM; **5**: EC_{50}= 110 nM; **6**: EC_{50}= 140 nM) were generally more effective for GPR34 receptor activation as compared with the corresponding regioisomeric 2°-acyl-1°-phosphoserine analogues (**7**-**12** except **12**) (**7**: EC_{50}= NA (not available due to weak activity); **8**: EC_{50}= NA: **9**: EC_{50} = >1.0 μM; **10**: EC_{50} > 1.0 μM; **11**: EC_{50} > 1 μM; **12**: EC_{50}= 190 nM) regardless of *cis* or *trans* configuration and fatty acid chain (A), (B) or (C) (Figure 4 and Table 1). Futhermore, the 1°-acyl-2°-phosphoserine analogues (**1**–**6**) showed comparable or stronger activation potency toward GPR34 receptors, as compared with the prototype LysoPS (EC_{50} = 240 nM). This result suggested that the space-filling

Scheme 3. Synthesis of Olefin-*trans*-1°-acyl-2°-phosphoserine[a]



[a]Conditions: (a) 1*H*-tetrazole, CH_2Cl_2, THF, r.t.; TBHP, CH_2Cl_2, r.t., Ar. (b) Amberlyst-15, MeOH, r.t., 99%. (c) Oleoyl chloride, DMAP, CH_2Cl_2, r.t. (d) Diisopropylcarbodiimide, (B) or (C), *N*,*N*-dimethylaminopyridine, CH_2Cl_2, r.t. (e) TFA, r.t.

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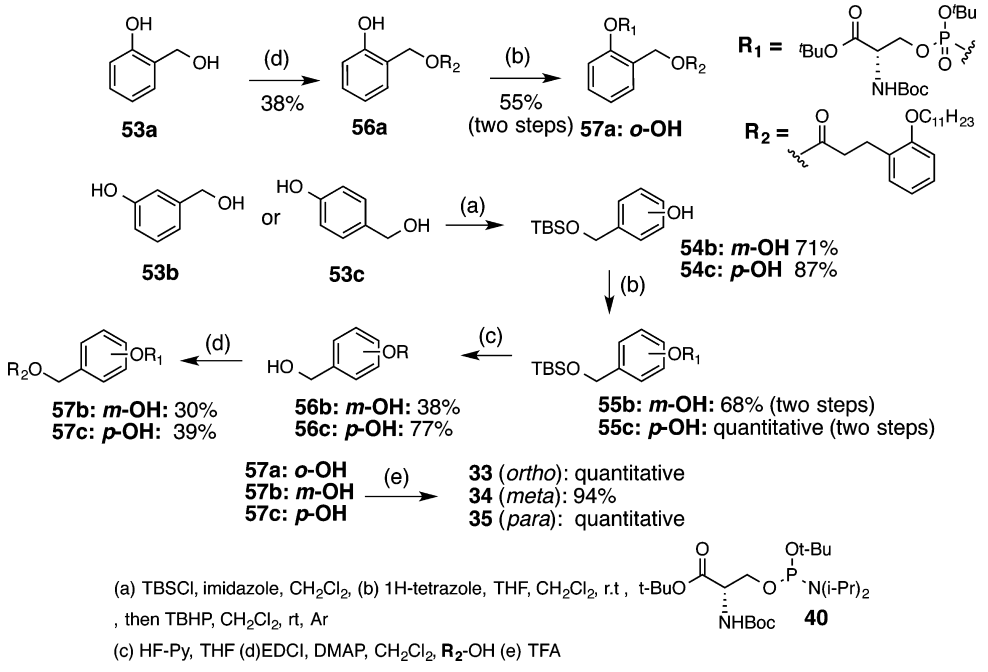
Scheme 4. Synthesis of Benzene-LysoPS Analogues



characteristics of the substructures of the 1°-acyl-2°-phosphoserine and 2°-acyl-1°-phosphoserine derivatives are different (Figure 9), and this difference may contribute to the receptor activation.

On the other hand, one set of the regioisomeric *cis*-derivatives (**6** and **12**) was rather inconsistent with the above trend: both 1°-acyl-2°-phosphoserine **6** and 2°-acyl-1°-phosphoserine **12** showed similar reactivity toward GPR34 activation (**6**: EC_{50}= 140 nM; **12**: EC_{50}= 190 nM for GPR34).

Moreover, *cis*- and *trans*-stereoisomerism influenced the potency of GPR34 activation: in the case of 1°-acyl-2°-phosphoserine analogues, *cis* isomers **5** and **6**, bearing acyl chains (B) and (C), respectively, exhibited more potent agonistic activity toward GPR34 than the corresponding *trans* isomers (**2** and **3**), while the derivatives bearing acyl chain (A), i.e., *trans* **1** and *cis* **4** showed a reverse order of activity toward GPR34.

It is noteworthy that the nonlipid type of acyl chain (C) is the most effective substructure for selectivity toward GPR34 over P2Y10 activation, as compared with the acyl chains (A) and (B) (Figure 4) (*vide infra*). Among these compounds (**3**, **6**, **9** and **12**), **6** and **12** showed the comparable agonistic selectivity toward GRR34, being approximately 100-fold more potent than that toward P2Y10. This result may indicate that the combination of *cis* configuration with acyl chain (C) affords a favorable overall molecular shape for high subtype selectivity (*vide infra*).

Activity toward P2Y10. All of the derivatives (**1**-**12**) showed very weak potency toward P2Y10 in comparison with endogenous LysoPS (EC_{50}= 25 nM). However, some of the derivatives bearing the acyl chains (A) and (B) showed distinct activity such as **1** (acyl chain (A)), **2**, **5**, and **8** (all bearing acyl chain (B)) (**1**: EC_{50}= 450 nM; **2**: EC_{50}= 610 nM; **5**: EC_{50}= 470 nM; **8**: EC_{50} = 290 nM for P2Y10 activation), even though the acivity is weaker than the original LysoPS. Intriguingly as pointed above, derivatives (**3**, **6**, **9** and **12**) bearing the acyl chain (C) diminished the activity toward P2Y10 significantly (**3**: EC_{50} > 1 μM; **6**: EC_{50} > 1 μM; **9**: EC_{50} > 1 μM; **12**: EC_{50} > 1 μM for P2Y10 activation). The conclusion that acyl chain (B) is the most favorable for P2Y10 activation is consistent with our previous SAR study.[18,19]

Activity toward GPR174. All the compounds (**1**-**12**) showed no significant activity toward GPR174, presumably because of deletion of the hydroxyl group at the *sn*-2 position of the glycerol backbone (Figure 4). As representative examples, the null activities of the derivatives **1**, **2**, and **3** were shown in Figure S4 (Supporting Information).

Effect of Fatty Acid C. In order to study further the reason for the equivalent effectiveness of 1°-acyl-type and 2°-acyl-type derivatives containing fatty acid C, i.e., cis-1°-(C)-2°-phosphoserine (**6**) and cis-2°-(C)-1°-phosphoserine (**12**) analogues, toward GPR34, we synthesized additional LysoPS analogues (**13**−**16**) bearing a substituent on the terminal benzene ring of acyl chain (C) (Figure 5), i.e., we introduced methyl and chlorine substituents on the terminal benzene ring of acyl chain (C) (Figure 5). As shown in Table 1, the activities toward GPR34 of the analogues containing a para-substituted benzene ring with acyl chain (C), cis-2°-(C)-1°-phosphoserine analogues **15** and **16**, were retained, and were comparable to or slightly weaker than those of cis-1°-(C)-2°-phosphate analogues **13** and **14** (**13**: EC_{50} = 37 nM; **14**: EC_{50} = 36 nM; **15**: EC_{50} = 120 nM; **16**: EC_{50} = 77 nM for GPR34). These activities are stronger than those of the unsubstituted acyl chain (C) derivatives (**6** and **12**). These results suggest a consistent trend of activity toward GPR34 with respect to regioisomerism of the acyl chain (C)-substituted derivatives. Furthermore, these derivatives did not show significant activity toward P2Y10 (**13**: EC_{50} > 1 μM; **14**: EC_{50} > 1 μM; **15**: EC_{50} > 1 μM; **16**: inactive for P2Y10) and therefore derivatives **13** and **14** showed the most potent and selective agonistic activity toward GRR34, being approximately 10-fold more potent than LysoPS itself.

The above results encouraged us to examine the most effective shape of fatty acid (C) in *cis*-1°-(C)-2°-phosphoserine (**6**) and *cis*-2°-(C)-1°-phosphoserine (**12**) analogues (Figure 6). According to the shedding assay results in Table 1, meta (**17** and **19**) and para isomers (**18** and **20**) of acyl chain (C) were

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Table 1. Agonistic Activities of Analogues Studied in This Work[a,b]

	GPR34	P2Y10
	EC50	
	(LogEC50)[c]	
	[Emax][c]	
	<RIA>[d]	
	number of data (n)[c]	
Positive Control	140 nM[d]	1.3 nM[d]
	(-6.86 ± 0.06)	(-8.88 ± 0.07)
	[14.8 ± 0.6%]	[10.6 ± 0.6%]
	<1>	<1>
	12	9
18:1-LysoPS	240 nM	25 nM
	(-6.62 ± 0.06)	(-7.61 ± 0.06)
	[9.5 ± 0.8%]	[12.5 ± 0.9%]
	<0.33 ± 0.05>	<0.073 ± 0.013>
	11	10
1	100 nM	450 nM
	(-7.00 ± 0.19)	(-6.34 ± 0.09)
	[8.7 ± 0.7%]	[3.9 ± 1.0%]
	<1.03 ± 0.31>	<0.0014 ± 0.0002>
	6	4
2	640 nM	610 nM
	(-6.19 ± 0.21)	(-6.22 ± 0.13)
	[5.3 ± 0.9%]	[7.5 ± 1.2%]
	<0.082 ± 0.023>	<0.0020 ± 0.0002>
	4	4
3	340 nM	> 1 μM
	(-6.46 ± 0.04)	(> -6)
	[9.8 ± 0.5%]	[NA]
	<0.24 ± 0.02>	<NA>
	8	4
4	210 nM	NA
	(-6.68 ± 0.08)	NA
	[11.1 ± 0.8%]	[<3%]
	<0.53 ± 0.05>	<NA>
	9	4
5	110 nM	470 nM
	(-6.96 ± 0.09)	(-6.33 ± 0.13)
	[5.2 ± 0.7%]	[10.6 ± 1.2%]
	<0.41 ± 0.04>	<0.0038 ± 0.0006>
	5	4
6	140 nM[e]	> 1 μM
	(-6.86 ± 0.06)	(> -6)
	[14.8 ± 0.6%]	[NA]
	<1>	<NA>
	12	4
7	NA	NA
	(NA)	(NA)
	[NA]	[NA]
	<NA>	<NA>
	3	3
8	NA	290 nM
	(NA)	(-6.54 ± 0.36)
	[<3%]	[7.2 ± 1.3%]
	<NA>	<0.0098 ± 0.0079>
	4	4
9	> 1 μM	> 1 μM
	(> -6)	(> -6)
	[NA]	[NA]
	<NA>	<NA>
	8	4
10	>1 μM	> 1 μM
	(> -6)	(> -6)
	[NA]	[NA]
	<NA>	<NA>
	4	6
11	> 1 μM	> 1 μM
	(> -6)	(> -6)
	[NA]	[NA]
	<NA>	<NA>
	4	3
12	190 nM	> 1 μM
	(-6.73 ± 0.10)	(> -6)
	[17.7 ± 1.6%]	[NA]
	<0.85 ± 0.15>	<NA>
	8	4
13	37 nM	> 1 μM
	(-7.44 ± 0.09)	(> -6)
	[16.6 ± 1.1%]	[NA]
	<3.8 ± 0.3>	<NA>
	8	4
14	36 nM	> 1 μM
	(-7.45 ± 0.04)	(> -6)
	[15.2 ± 0.8%]	[NA]
	<3.6 ± 0.3>	<NA>
	8	4
15	120 nM	> 1 μM
	(-6.93 ± 0.03)	(> -6)
	[15.1 ± 1.1%]	[NA]
	<1.2 ± 0.2>	<NA>
	6	5
16	77 nM	NA
	(-7.12 ± 0.06)	NA
	[16.0 ± 1.0%]	[NA]
	<1.9 ± 0.3>	<NA>
	6	4
17	> 1 μM	> 1 μM
	(> -6)	(> -6)
	[NA]	[NA]
	<NA>	<NA>
	3	3
18	NA	> 1 μM
	(NA)	(> -6)
	[NA]	[NA]
	<NA>	<NA>
	3	3
19	NA	> 1 μM
	(NA)	(> -6)
	[NA]	[NA]
	<NA>	<NA>
	3	3
20	> 1 μM	> 1 μM
	(> -6)	(> -6)
	[NA]	[NA]
	<NA>	<NA>
	3	3
21	> 1 μM	450 nM
	(> -6)	(-6.34 ± 0.17)
	[NA]	[11.5 ± 1.7%]
	<NA>	<0.004± 0.0016>
	3	3
22	NA	17 nM
	(NA)	(-7.77 ± 0.03)
	[<3%]	[7.6 ± 0.8%]
	<NA>	<0.071 ± 0.013>
	5	5
23	460 nM	330 nM
	(-6.33 ± 0.20)	(-6.49 ± 0.07)
	[11.7 ± 2.3%]	[11.5 ± 0.5%]
	<0.25 ± 0.04>	<0.0053 ± 0.0002>
	3	3
24	> 1 μM	> 1 μM
	(> -6)	(> -6)
	[NA]	[NA]
	<NA>	<NA>
	3	3
25	510 nM	42 nM
	(-6.29 ± 0.15)	(-7.38 ± 0.10)
	[5.0 ± 1.7%]	[12.4 ± 0.5%]
	<0.086 ± 0.018>	<0.050 ± 0.011>
	4	5

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Table 1. continued

	GPR34	P2Y10
26	600 nM	> 1 μM
	(-6.22 ± 0.08)	(> -6)
	[12.4 ± 1.8%]	[NA]
	<0.20 ± 0.02>	<NA>
	4	3
27	NA	> 1 μM
	(NA)	(> -6)
	[NA]	[NA]
	<NA>	<NA>
	3	3
28	NA	> 1 μM
	(NA)	(> -6)
	[NA]	[NA]
	<NA>	<NA>
	3	3
29	> 1 μM	> 1 μM
	(> -6)	(> -6)
	[NA]	[NA]
	<NA>	<NA>
	3	3
30	560 nM	> 1 μM
	(-6.26)	(> -6)
	[3.8 %]	[NA]
	<0.089>	<NA>
	3	3
31	> 1 μM	> 1 μM
	(> -6)	(> -6)
	[NA]	[NA]
	<NA>	<NA>
	3	3
32	180 nM	> 1 μM
	(-6.74 ± 0.14)	(> -6)
	[16.5 ± 2.4%]	[NA]
	<0.86 ± 0.10>	<NA>
	4	4
33	700 nM	45 nM
	(-6.15 ± 0.17)	(-7.35 ± 0.13)
	[5.9 ± 1.3%]	[5.8 ± 0.7%]
	<0.20 ± 0.13>	<0.020 ± 0.003>
	5	5
34	NA	6.7 nM
	(NA)	(-8.18 ± 0.09)
	[NA]	[10.8 ± 0.8%]
	<NA>	<0.23 ± 0.06>
	3	9
35	NA	> 1 μM
	(NA)	(>-6)
	[NA]	[NA]
	<NA>	<NA>
	3	3

[a]Activities are represented in terms of EC_{50} (see Experimental Section). [b]"NA" means "not available" because of very low activity. [c]Log EC_{50} (M) and E_{max} (% AP-TGFα release) values are calculated from a sigmoidal concentration−response curve (see Experimental Section) and shown as Mean ± SEM of indicated numbers of independent experiments (n). EC_{50} values are calculated from a mean value of LogEC_{50}. [d]RIA (relative intrinsic activity; a dimensionless parameter; ref 24) is an estimation of agonist activity and represented as a E_{max}/EC_{50} value relative to that of a reference compound (**6** for GPR34 and a compound **10b** (diF-C3-ph-o-O-C11) in the previous work (ref 19) for P2Y10). By definition, the RIA value of a reference compound is equal to 1. EC_{50} values are calculated from a mean value of LogEC_{50}.

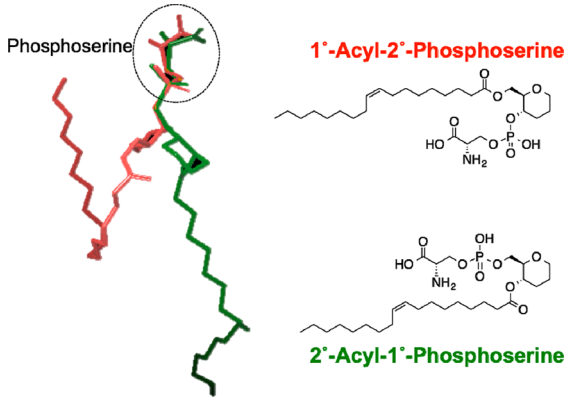

Figure 9. Different space-filling characteristics of substructures of cyclic LysoPS analogues, *trans* 1°-acyl-2°-phosphoserine (**1**) vs *trans* 2°-acyl-1°-phosphoserine (**7**). Representative structures evaluated by conformation search calculations.

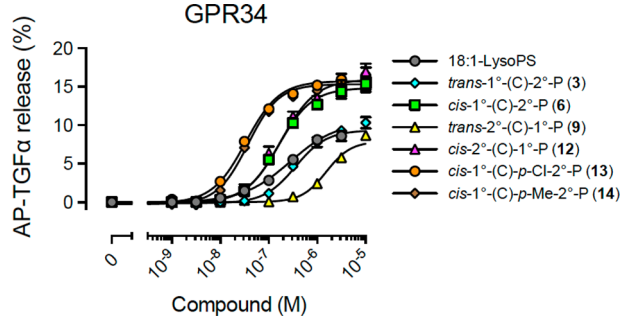

Figure 10. LysoPS analogues differentially activate GPR34. HEK293 cells transiently transfected with GPR34-encoding expression vector or an empty vector were treated with compounds and receptor-specific AP-TGFα release responses were determined by subtracting background responses in the empty vector-transfected cells (actual signals in the GPR34-expressing cells and the empty vector-transfected cells are shown in Supporting Information Figure S2). Data are mean and SEM (standard error of the mean) for seven to 11 independent experiments.

inactive toward GPR34 (**17**: EC_{50} > 1 μM; **18**: inactive; **19**: inactive; **20**: EC_{50} > 1 μM for GPR34), while ortho compounds **6** and **12** are potent activators of GPR34 (Figure 6). This suggests that an ortho-substituted propionic acid skeleton is important for GPR34 activation. Furthermore, derivatives **17**-**20** did not show significant activity for P2Y10 at all (**17**: EC_{50} > 1 μM; **18**: EC_{50} > 1 μM; **19**: > 1 μM; **20**: EC_{50}> 1 μM for P2Y10).

In the present combinations of 1-acyl/2-acyl and *cis/trans* isomerisms of the 2-hydroxymethyl-3-hydroxytetrahydropyran ring system, the concentration−response data of the resultant potent derivatives (**6**, **12**, **13**, **14**) is shown in Figure 10, together with LysoPS and some less active counterparts (**3** and **9**).

Effect on Intracellular Calcium Mobilization in HEK293-GPR34 Cells. We further examined whether the active compounds found by the TGFα shedding assay (*cis*-1°-acyl-2°-phosphate analogues **6**, **13**, and **14** and *cis*-2°-acyl-1°-phosphate analogues **12**, **15**, and **16**) induced a transient increase in an intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) through activation of GPR34 (Figure 11). The compounds (**6**, **13**, **14**, **12**, **15**, and **16**) induced robust Ca^{2+} responses in the cells expressing GPR34 and the chimeric $G\alpha_{q/i1}$ subunit. The responses were completely absent in cells expressing the chimeric $G\alpha_{q/i1}$ subunit alone (mock cells; data not shown). The data clearly showed that the compounds **6** and **12**−**16** induced a transient increase in $[Ca^{2+}]_i$, which is consistent with

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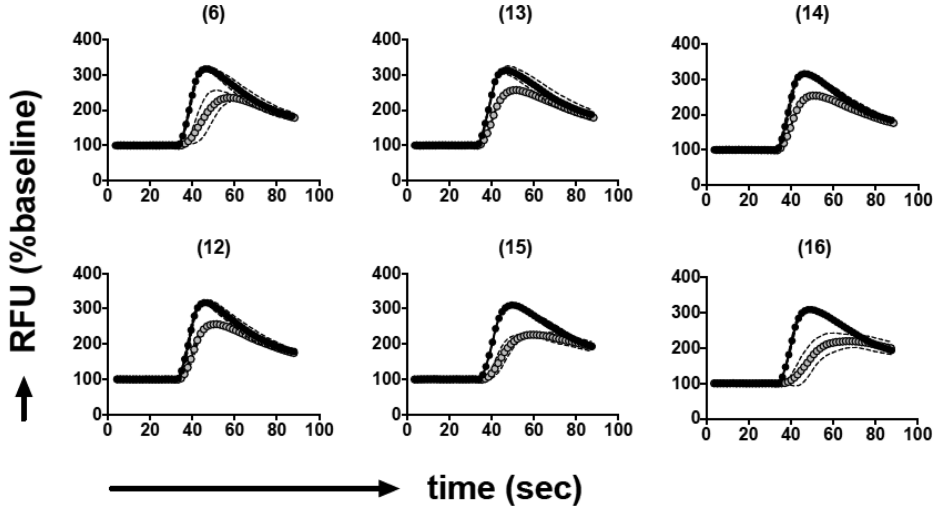

Figure 11. Effects of shedding-assay-positive compounds on intracellular calcium mobilization ($[Ca^{2+}]_i$) in HEK293-GPR34 cells. Calcium 5 dye-loaded HEK293 cells transiently expressing GPR34 together with the chimeric $G\alpha_{q/i1}$ subunit were stimulated with 1 μM compound (black symbol) or 100 nM compound (gray symbol), and ($[Ca^{2+}]_i$) was measured fluorometrically using a FlexStation 3 (Molecular Devices) liquid handling microplate reader (Molecular Devices). Dotted lines, SD of triplicate measurements of a single assay. A test compound was added at approximately 35 s from the initial measurement. Note that there was not significant $[Ca^{2+}]_i$ in the negative-control cells expressing the $G\alpha_{q/i1}$ subunit alone (not shown).

activation of GPR34 (Figure 11). The effects of other compounds (**1**−**35**) on a transient increase in $[Ca^{2+}]_i$ were compiled in Supporting Information (Figures S5 and S6).

Analysis of Solution Conformations of Saturated 2-Hydroxymethyl-3-hydroxytetrahydropyran Derivatives. We examined the conformations of the saturated 2-hydroxymethyl-3-hydroxytetrahydropyran derivatives in solution by using NMR spectroscopy. Although it would be preferable to examine the conformation of whole molecule including the acyl chain and phosphoserine (Figure 4), this was not feasible due to the broadness and overlapping of the ^{1}H NMR signals. Therefore, we studied the conformation of several model compounds including saturated **76** (*trans*), **77** (*cis*), containing bulky TBS protecting group on the primary alcohol, and **78** (*trans*), **79** (*cis*), composed of bulky protected phosphoserine unit on the secondary alcohol, in two different solvents, $CDCl_3$ and CD_3OD (Figure 12) (Supporting Information Figure S7).

We successfully detected major conformation of each cyclic scaffolds of model saturated **76** (*trans*) and **77** (*cis*) compounds (Figure 12A) by means of NOESY spectra. That is, in the case of a saturated trans-configuration (like **76**), the large substituent on the primary position and the hydroxyl group on the secondary position are located in diequatorial positions (**76-*ee***) rather than in diaxial positions (**76-*aa***), reasonably because the diequatorial conformation is the most favorable to avoid steric hindrance generated by 1,3-diaxial interactions between the methylene moiety at the primary oxygen and the diaxial H_3 and H_5 protons (Figure 12A). In solution phase a single conformation can be detected. In the case of *cis*-configuration (like **77**), the NOESY experiments suggested that the primary substituent prefers to exist in the equatorial position and the hydroxyl group on secondary position likely to be on axial position (**77-*ea***), rather than the conformation (**77-*ae***) in which the former in the axial position and the latter in the equatorial position, due to avoiding 1,3-diaxial interaction between the methylene moiety of the primary position and diaxial H_3 and H_5 protons.

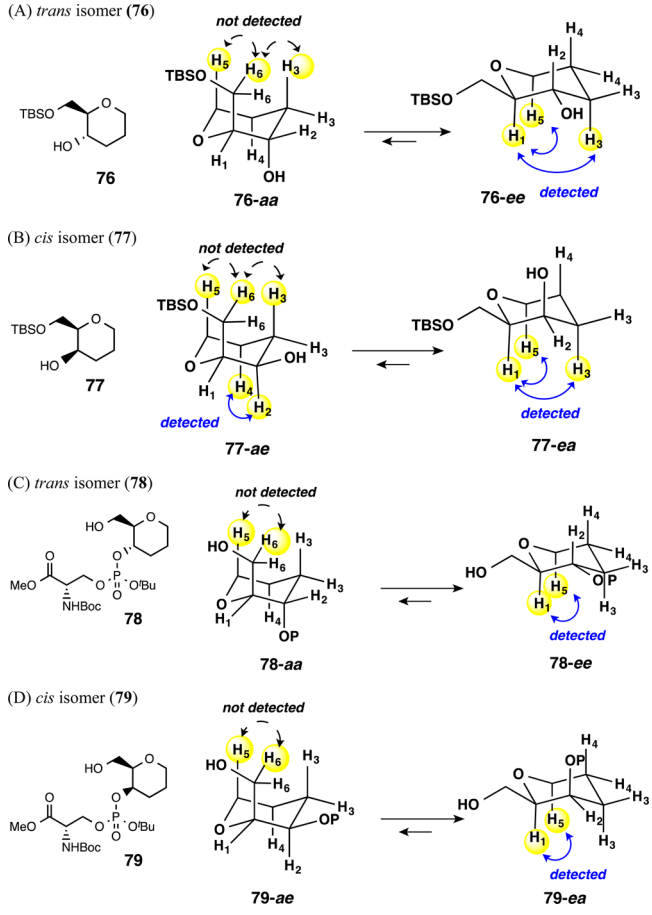

Figure 12. Conformational equilibrium of the (A) *trans* isomer (**76**) and (B) *cis* isomer (**77**) of 2-hydroxymethyl-3-hydroxytetrahydropyran models substituted with a TBS group on the primary position; and of the (C) *trans* isomer (**78**) and (D) *cis* isomer (**79**) of substituted phosphorserine on the secondary position.

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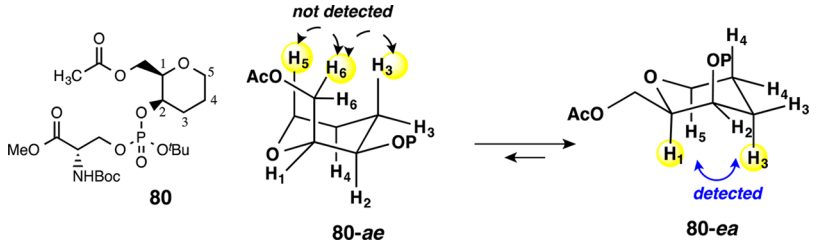

Figure 13. Conformational equilibrium of the *cis* isomer of 2-hydroxymethyl-3-hydroxytetrahydropyran models (**80**), which has substituents on both the primary and secondary positions.

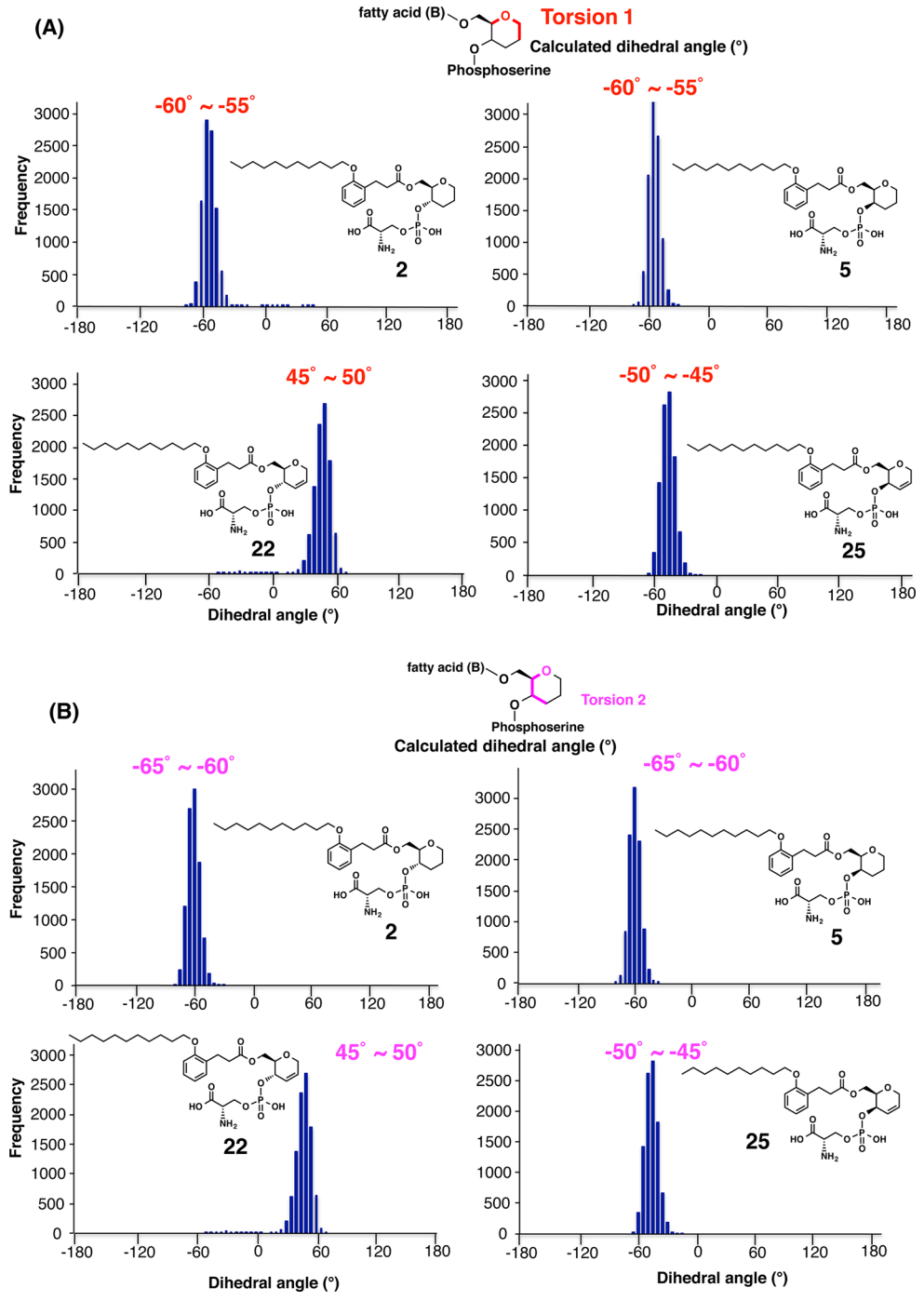

Figure 14. Distributions of the dihedral angles of the tetrahydropyran ring in calculated ring structures which are obtained by molecular dynamic simulation, indicating the effect of increased ring planarity arising from replacement of tetrahydropyran with a dihydropyran moiety: (A) torsion angle 1; (B) torsion angle 2.

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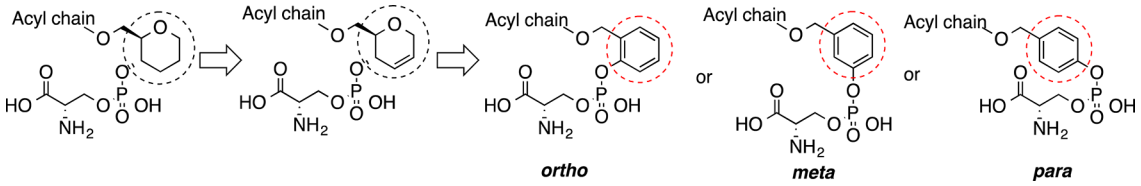

Figure 15. Morphing the glycerol moiety into a benzene ring.

We also studied the conformation of model saturated compounds **78** (*trans*) and **79** (*cis*) which contain a protected phosphserine moiety on the secondary alcohol, in $CDCl_3$ and CD_3OD (Figure 12C and D). The NMR analysis indicated that a single conformation was favored in **78-*ee*** and **79-*ea***, similar to that of the former model compounds (**76-*ee*** and **77-*ea***), which contain a TBS group on the primary position.

Finally, we studied the more close model compound **80** (*cis*) via NMR spectroscopy, which has an acetyl group on the primary alcohol and the protected phosphserine moiety on the secondary alcohol (Figure 13). In this *cis* configuration, a single major conformation can be detected in the NMR spectra; that is, the acyl subsituent on the primary position is favorable to be in the equatorial position and the protected phosphserine group on the secondary position is likely to be in the axial position. This conformational preference probably resulted from the steric hindrance between the methylene substituent on the primary position and the H_3 and H_5 protons expected in the alternative conformation (Figure 13). The observed conformational preference of saturated model compounds **78** and **79** coincided with those found in saturated models **76** and **77**, indicating the presence of conformational bias independent of the O-subsitutents.

Introduction of a 2-hydroxymethyl-3-hydroxytetrahydropyran skeleton (**b**, Figure 2) in place of the glycerol moiety (**a**, Figure 2) indeed restricts the conformation of the glycerol moiety, leading to restruction of the extension of fatty acid and phosphoserine moieties. The overall shape of the molecules may be suitable for GPR34 binding, rather than for P2Y10 binding.

Effect of Unsaturation of the Tetrahydropyran Framework on Receptor Activation. The synthesized dihydroyran-type LysoPS analogues (**c** in Figure 2, **21**−**32** in Figure 7) were evaluated for agonistic activity toward GPR34 and P2Y10 (Table 1). Interestingly, unsaturated cyclic LysoPS analogues also have similar activity tendency to the saturated cyclic LysoPS analogues case, that is, 1-acyl-2-phosphoserine analogues (**21**−**26** except **26**) (**21**: EC_{50} > 1 μM; **22**: EC_{50} = very weak; **23**: EC_{50} = 460 nM; **24**: EC_{50} > 1 μM; **25**: EC_{50} = 510 nM; **26**: EC_{50} = 600 nM for GPR34) generally showed higher potency toward GPR34 than their regioisomers, the 2-acyl-1-phosphoserine analogues (**27**−**32** except **32**: **27**: inactive; **28**: inactive; **29**: EC_{50} > 1 μM; **30**: EC_{50} 560 nM: **31**: EC_{50} > 1 μM; **32**:EC_{50} = 180 nM for GPR34), with improved selectivity. In addition, in the 1-acyl-2-phosphoserine topology the potency of the unsaturated analogues (**21**-**26**) for GPR34 activation was reduced as compared with the saturated analogues (**1**−**6**). The *trans* isomers (**21**−**23**) of 1-acyl-2-phosphoserine derivatives showed activity comparable to the *cis* isomers (**24**−**26**) for GPR34 activation.

In particular, the dihydropyran analogues (**c** in Figure 2, **21**−**26** in Figure 7) showed more potent agonistic activities against P2Y10 than the corresponding saturated analogues (**1**−**6**), and the *trans*-unsaturated analogues (**21**−**23**) were more potent than the *cis*-unsaturated analogues (**24**−**26**): (**21**: EC_{50} = 450 nM; **22**: EC_{50} = 17 nM; **23**: EC_{50} = 330 nM; **24**: EC_{50} > 1 μM; **25**: EC_{50} = 42 nM; **26**: EC_{50} > 1.0 μM for P2Y10) (Table 1). On the other hand, the 2°-acyl-1°-phosphoserine analogues (**27**−**32**) did not show significant P2Y10 activity (**27**: EC_{50} > 1 μM; **28**: EC_{50} > 1 μM; **29**: EC_{50} > 1.0 μM; **30**: EC_{50} > 1 μM; **31**: EC_{50} > 1 μM; **32**: EC_{50} > 1 μM for P2Y10). The implication that acyl chain (B) is a favorable substructure for P2Y10 activation is consistent with the previous SAR study,[19] in which 1°-acyl-type analogues, *trans*-isomers **21** ((**A**)), **22** ((**B**)) and **23** ((**C**)), and *cis*-isomers **24** ((**A**)), **25** ((**B**)) and **26** ((**C**)), were compared. We hypothesized that these changes in subtype selectivity can be interpreted in terms of increased ring planarity of the dihydropyran ring moiety due to the introduction of a double bond into the tetrahydropyran ring, based on the distribution of accessible dihedral angles in the calculated ring structures. That is, the respective disributions of two dihedral angles (torsion 1 (**A**) amd torsion 2 (**B**)) of unsaturated analogues **22** (*trans*) and **25** (*cis*) decreases in the absolute magnitude as comapred with those of the saturated counterparts (**2** (*trans*) and **5** (*cis*), respectively), supporting the above interpretaion (Figure 14). Consequently, we next aimed to replace the dihydropyran moiety (**c**, Figure 2) with a benzene ring (**d**, Figure 2) in order to further investigate the effect of ring planarity on the agonistic activity.

Aromatized Glycerol Mimics. We synthesized analogues in which the tetrahydropyran framework was replaced with a benzene ring and examined their agonistic activities (Figure 15).

Three kinds of benzene-type LysoPS analogues, *ortho* (**33**), *meta* (**34**), and *para* (**35**), can be generated (Figure 8 and Scheme 4). The agonistic activities of the synthesized aromatic LysoPS analogues were evaluated, and the results are shown in Table 1.

Replacement of glycerol (**a**) with a benzene ring (**d**) increased the potency toward P2Y10, as compared with the saturated (**b**) and unsaturated tetrahydropyran (**c**) analogues (Figure 2). While the *para*-analogue (**35**) shows significantly diminished activities at any receptor (**35**: inactive for GPR34; EC_{50} > 1 μM for P2Y10), the *ortho* (**33**) and *meta* isomers (**34**) showed potent and selective activation of P2Y10 (**33**: EC_{50} 700 nM for GPR34; EC_{50}: 45 nM for P2Y10; **34**: inactive for GPR34; EC_{50} = 6.7 nM for P2Y10). Comparison of saturated (**2**) and unsaturated cyclic analogues (**22**) and the *meta* isomer (**34**) showed that compounds **22** and **34**, which include increased planarity of the ring moiety, have more potent agonistic activities toward P2Y10 than that of compound **2**, which means that ring planarity is probably involved in the agonsitic activity for P2Y10 and GPR34 with potency and receptor selectivity. Among the results of the agonistic activities of benzene ring analogues, the *meta* isomer showed the most potent agonistic activity against P2Y10 with high selectivity. The results suggested that the positions of the substituents also has an influence on the acitivity change. According to this

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consideration, the substituents on the *meta* position (**34**) are the best framework of the benzene ring, which showed more potent agonistic activity against P2Y10 than that of the *ortho* and *para* isomers.

The present shape morphing the 2-hydroxymethyl-3-hydroxytetrahydropyran ring system into a benzene ring through the dihydropyran ring enhanced the P2Y10 activation potency rather than GPR34 activation. The concentration−response data of the resultant potent derivatives (**22**, **25**, **34**) is shown in Figure 16, together with LysoPS and some less active counterparts (**33** and **35**).

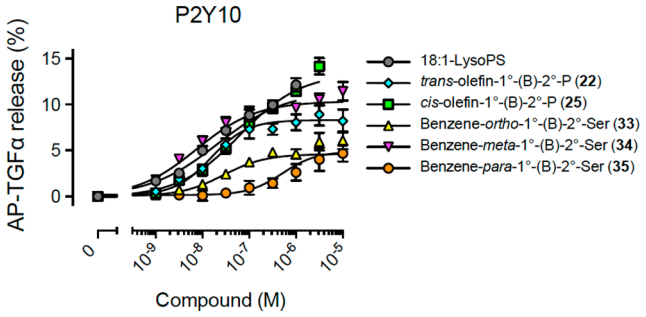

Figure 16. LysoPS analogues differentially activate P2Y10. HEK293 cells transiently transfected with P2Y10-encoding expression vector or an empty vector were treated with compounds, and receptor-specific AP-TGFα release responses were determined by subtracting background responses in the empty vector-transfected cells (actual signals in the P2Y10-expressing cells and the empty vector-transfected cells are shown in Supporting Information Figure S3). Data are mean and SEM (standard error of the mean) for three to ten independent experiments.

Actin Stress Fiber Formation Assay. To further confirm the potency of the present LysoPS analogues at the cellular level *in vitro*, we utilized another *in vitro* GPCR assay (actin stress fiber formation assay), in which P2Y10-induced $G\alpha_{12/13}$ signaling is detectable as formation of actin stress fibers.[1] We tested P2Y10-selective agonists **22**, **33**, and **34**, the GPR34-selective agonist **6** and **12** in addition to pan-agonist LysoPS, GPR34 - P2Y10 dual agonist **25** and nonresponsive compound **35**. LysoPS induced formation of actin stress fibers in P2Y10-expressing cells (Figure 17). Among the tested compounds, **22**, **25**, **33**, and **34** induced formation of actin stress fibers, but **6**, **12**, and **35** did not. Actin stress fiber formation was not observed in stably mock-infected cells (Supporting Information, Figure S8). These results further establish the potency of these analogues and also support the generality of the characteristics of these LysoPS analogues found in the TGFα shedding assay.

CONCLUSION

We have discovered lysophosphatidylserine analogues containing the conformationally constrained glycerol that show potent and selective activity toward GPR34 and P2Y10. We established a synthetic route to 1°-acyl-2°-phosphoserine and 2°-acyl-1°-phosphoserine analogues by introducing a tetrahydropyran moiety, which has a nonequivalent hydroxyl group, to obtain a set of cyclic LysoPS analogues. 1°-Acyl-2°-phosphoserine analogues showed greater potency as receptor activators. In these compounds, the acyl chain (B) was effective for activity toward P2Y10 and the acyl chain (C) for activity toward GPR34, in accordance with our previous SAR study. Futhermore, all the derivatives (**1**−**35**) studied in this paper did not show significant activity toward GPR174. These results are consistent with the previous finding that this hydroxyl group is important for activation of GPR174.

Therefore, these cyclic LysoPS analogues are expected to be useful tools for selective modulation of GPR34 and P2Y10 in studies of the receptor functions. Further study of the configurations and space-filling conformations of these compounds may be useful in identifying recognition motifs and pharmacophores, and may also be helpful in future computational modeling and docking studies of GPR34 and P2Y10.

EXPERIMENTAL SECTIONS

Bioassay Based on Ectodomain Shedding of a Membrane-Bound Proform of Alkaline Phosphatase-Tagged TGFα. TGFα shedding assay was performed as described previously[11,12,17,18] with minor modifications. The TGFα shedding assay detects activation of GPCRs by measuring ectodomain shedding of a membrane-bound proform of alkaline phosphatase (AP)-tagged TGFα (AP-TGFα) into conditioned media. To monitor activation of G_i-coupled GPR34, we additionally transfected a chimeric $G\alpha_{q/i1}$ subunit to detect activation of G_i-coupled GPR34. We examined TGFα shedding response of synthetic compounds using mouse GPR34, P2Y10, and GPR174 LysoPS receptors.

We employed two different HEK293 cell lines (HEK293A and HEK293FT) because the responses induced by LysoPS in receptor-expressing cells were different among the LysoPS receptors.[18] That is, for GPR34 and P2Y10, HEK293A showed better responses than HEK293FT, and conversely, for GPR174, HEK293FT showed better responses.[18] As for the usage of LPA receptor antagonist (Ki16425, antagonist for LPA_1, LPA_2, and LPA_3), we have found that addition of Ki16425 reduced the background response in mock-transfected cells.[18] This is due to a possible contamination of a small amount of LPA or LPA-like compounds in LysoPS analog preparation or a possible conversion of LysoPS analogs to LPA-like compound after they are added to the cells (removal of serine moieties from LysoPS-like analogs by lysophospholipase D activity results in production of LPA-like molecules). HEK293 cells endogenously express LPA_1, LPA_2 and LPA_3 and presence of Ki16425 in the assay condition could diminish effects of LPA-like molecules on signals mediated by the endogenous LPA receptors.

Briefly, transfection was performed in a 100 mm culture dish using the following combination of a cell line and expression vector plasmids: for GPR34, HEK293A cells with a mixture of plasmids (AP-TGFα (2.5 μg), mouse GPR34 (2.5 μg), and chimeric $G\alpha_{q/i1}$ subunit (0.5 μg)) using 20 μL of 1 mg/mL Polyethylenimine Max solution (Polysciences); for P2Y10, HEK293A cells with a mixture of plasmids (AP-TGFα (2.5 μg) and mouse P2Y10 (2.5 μg)) using Lipofectamine 2000 reagent (Thermo Fisher Scientific); for GPR174, HEK293FT cells with a mixture of plasmids (AP-TGFα (2.5 μg) and mouse GPR174 (2.5 μg)) using 20 μL of the Polyethylenimine Max solution. Twenty-four h after transfection, cells were detached, suspended in 5 mM HEPES (pH 7.4)-containing Hanks' Balanced Salt Solution (HBSS) and seeded (80 μL per well) in a half-area 96-well plate in which serial concentrations of compounds (10 μL per well; diluted with 0.01% (w/v) bovine serum albumin-containing HBSS) and 100 μM Ki16425 (LPA_1 and LPA_3 inhibitor; 10 μL per well; diluted with HBSS) were predispensed (total conditioned media volume of 100 μL per well). After 1.5-h incubation, conditioned media (80 μL per well) were transferred to a blank, half-area 96-well plate. *p*-nitrophenyl phosphate solution was added and absorbance at wavelength of 405 nm (OD_{405}) was measured using a microplate reader (SpectraMax 340 PC384, Molecular Devices) before and after 1-h incubation at room temperature.

To calculate GPCR-mediated AP-TGFα shedding responses of compounds, raw absorbance data were processed as below (also see the Supporting Information Figure S1): (1) calculate increase of OD_{405} values before and after 1-h incubation, (2) calculate percentage

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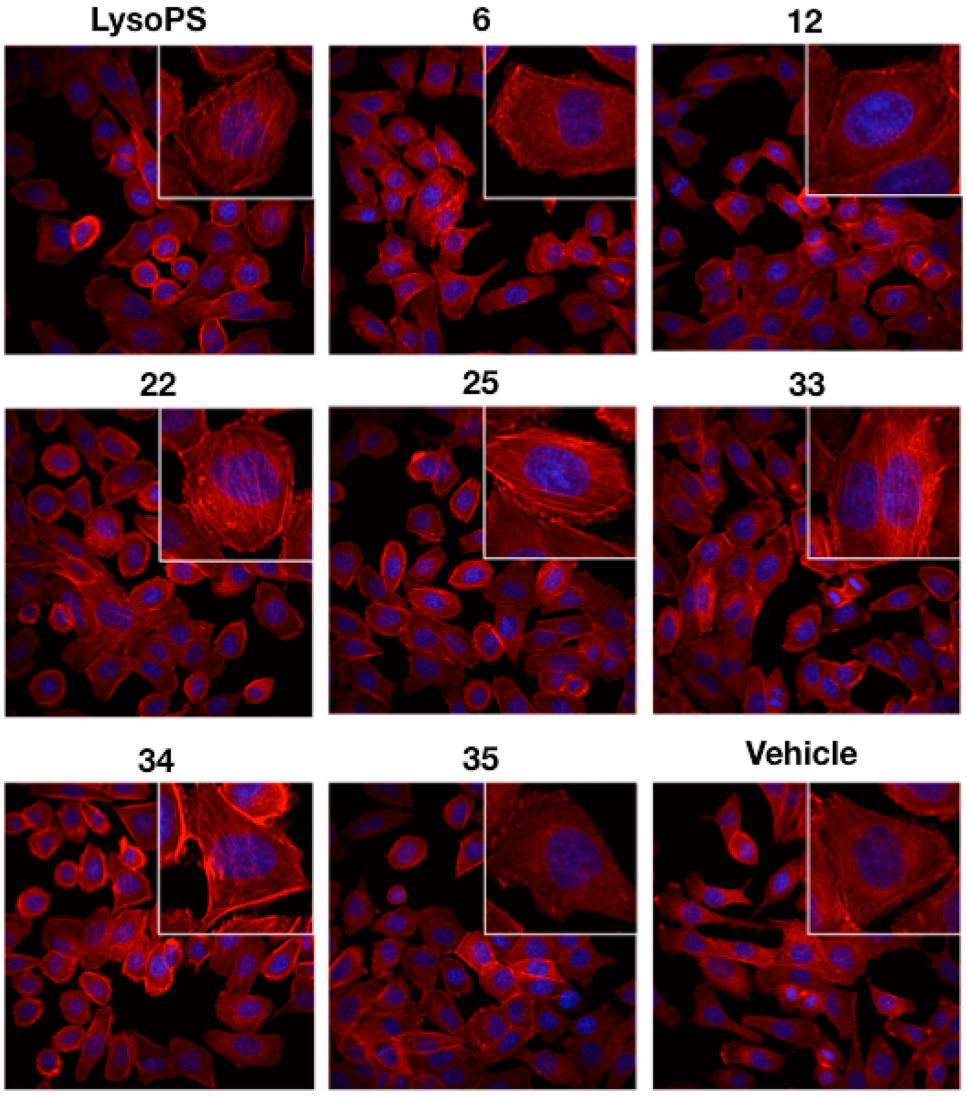

Figure 17. Formation of actin stress fibers in P2Y10-expressing cells induced by P2Y10 agonists. McA-RH7777 cells stably expressing mouse P2Y10 or stably infected with an empty retrovirus (control) were serum-starved and treated with or without 100 nM compounds for 30 min. The cells were fixed and stained with Alexa 594-labeled phalloidin (filamentous actin, red) and DAPI (nuclei, blue). Images were obtained with the IN Cell Analyzer 2000 (GE Healthcare) fluorescent imaging system. An inlet in each panel shows an enlarged view of a single cell. Note that the P2Y10-dependent fiber-like pattern of phalloidin staining was visible in an intracellular region in cells stimulated with LysoPS, **22**, **25**, **33**, or **34**. No significant fiber-like pattern of phalloidin staining was visible in mock cells (Figure S7).

of OD_{405} values in conditioned media (AP-TGFα_{CM}; relative amount of cleaved AP-TGFα in conditioned media), (3) subtract a vehicle-treated AP-TGFα_{CM} value form compound-treated AP-TGFα_{CM} values (AP-TGFα release; compound-induced response from a baseline (spontaneous) AP-TGFα shedding response), (4) subtract AP-TGFα release in mock-transfected cells from that in GPCR-expressing cells (Receptor-specific AP-TGFα release). A four-parameter sigmoid curve was fitted to concentration−response plots (typically 10 concentration points) using GraphPad Prism 6 (GraphPad, USA) and $LogEC_{50}$ and E_{max} values were computed for active compounds with plateau or semiplateau responses.

A detailed description of the compound preparation and administration for the biological assay is as follows: we dissolved a synthesized compound (a powder form) in 0.1% (w/v) bovine serum albumin (BSA; protease- and fatty acid-free grade from SERVA)-containing Dulbecco's phosphatase-buffered saline (D-PBS) at a concentration of 1 mM with repeated vortexes and water-bath sonication. The dissolved compound solution was aliquoted and stored at −25 °C. On the day of the TGFα shedding assay, we thawed frozen aliquots of compound solutions and diluted the solution using 0.01% (w/v)-containing Hank's balanced salt solution supplemented with 5 mM HEPES (pH 7.4). The serially diluted solution (3.2-fold dilution starting from 320 μM, which corresponds to a final concentration of 32 μM) was used as 10X concentration of test compounds. We predispensed 10 μL (per well in a 96-well plate) of 100 μM Ki16425, antagonist for LPA_1, LPA_2 and LPA_3, diluted with 5 mM HEPES (pH 7.4)-containing HEPES. We seeded 80 μL of cell suspension and treated with 10 μL of 10X test compound (also see below). For an initial experiment, we added test compounds to cells transfected with the AP-TGFα plasmid and the pCAGGS empty vector (mock-transfected cells) and measured nonspecific AP-TGFα release signal. For each compound, we chose highest concentration (32 μM, 10 μM, 3.2 μM or 1 μM at a final concentration) that did not show high nonspecific AP-TGFα release signal in the mock-transfected cells, and used the highest concentration for the subsequent assay for measuring LysoPS receptor activity.

In Figures 10 and 16, we did not include data points at 10 μM concentration for LysoPS(18:1) and **25**. We often observed that high concentrations of compounds damaged cells owing to their detergent-like effects and caused artificial signals even in mock-transfected cells. Thus, at these high concentrations, we could not correctly measure receptor activation. The membrane-damaging effects depend on

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physical property of each compound. In practice, we initially tested effects of test compounds on mock-transfected cells and determined the concentration at which test compounds induced such artifacts. We then chose the highest concentrations at which we would use for following experiments using receptor-expressing cells. For example, 18:1 LysoPS and the compound **25** at 10 μM induced artificial signals and thus we omitted testing at that concentration in the following experiments. In the present work, we focused on development of highly active agonists and, even in the absence of the data point at 10 μM, we could successfully determine saturable concentration−response curves for them.

Ca^{2+} Mobilization Assay. HEK293A cells were seeded in culture medium (DMEM supplemented 10% fetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin) in 100 mm culture dishes and incubated for 24 h. Then, mouse GPR34-expressing pCAGGS plasmid (5 μg per one 100 mm dish) and $G\alpha_{q/i1}$-expressing pCAGGS plasmid (2.5 μg) were transfected in HEK293A cells using Lipofectamine 2000 reagent (Thermo Fisher Scientific; 12.5 μL) according to manufacturer's protocol. For negative-control (mock) cells, empty pCAGGS plasmid (5 μg) and the $G\alpha_{q/i1}$-expressing pCAGGS plasmid (2.5 μg) were transfected. After incubation for 24 h, the cells were detached with 0.05% (w/v) trypsin containing 0.52 mM EDTA. After centrifugation, cells were suspended at a cell concentration of 5×10^5 cells/mL in serum-free DMEM, and seeded (50 μL per well) in CELLSTAR Advanced TC half a rea 96-well black plates (Greiner Bio-One). The plates were placed in an incubator for 24 h. A Ca^{2+} indicator (FLIPR Calsium 5 Assay Kit; Molecular Devices) was added (50 μL per well) and loaded into cells with 1-h incubation in the presence of 2.5 mM probenecid according to manufacturer's protocol. Then, the cell plate and a compound plate containing 5X test compounds were positioned in a fluorescence microplate reader (FlexStation 3; Molecular Devices) and fluorescent signal was measured with automated pipetting (25 μL of 5X compounds per well).

Actin Stress Fiber Formation Assay. Actin stress fiber formation assay was performed as described previously (1). McA-RH7777 rat hepatoma cells stably expressing mouse P2Y10 or mock-infected cells were seeded in a collagen type I coated 96-well plate at 2.5×10^4 cells per well and incubated for 24 h in culture medium (DMEM supplemented with 10% (v/v) FBS). The cells were washed once with PBS and serum-starved for 1 h in serum-free, 0.01% (w/v) BSA (essentially fatty acid-free grade)-containing DMEM. The cells were then stimulated with LysoPS (1) or its analogues at a concentration of 100 nM for 30 min. The cells were washed with PBS and fixed with 4% (w/v) paraformaldehyde-containing PBS for 20 min. After brief washing with PBS, the cells were permeabilized with 0.1% (v/v) Triton X-100-containing PBS and stained with Alexa Fluor 594-conjugated phalloidin (for filamentous actin) at 1.5 μg/mL and 4′,6-diamidino-2-phenylindole (DAPI; for nuclei) at 1 μg/mL in 3% (w/v) BSA-PBS for 1 h. The cells were washed with PBS and fluorescence images were acquired using a fluorescent microscopy (IN Cell Analyzer 2000, GE Healthcare). The two channels were pseudocolored (red for phalloidin and blue for DAPI) and shown as a merged image.

Chemical Synthesis. *General Procedures.* Melting points were determined with a Yanaco micro melting point apparatus without correction. ^{1}H- (400 MHz) and ^{13}C- (100 MHz) NMR spectra were recorded on a Bruker Avance400. Chemical shifts were calibrated with tetramethylsilane as an internal standard or with the solvent peak, and are shown in ppm (δ) values, and coupling constants are shown in hertz (Hz). The following abbreviations are used: s = singlet, d = doublet, t = triplet, q = quartet, dd = double doublet, dt = double triplet, dq = double quartet, h = hextet, m = multiplet, and brs = broad singlet. Electron spray ionization time-of-flight mass spectra (ESI-TOF MS) were recorded on a Bruker micrOTOF-05 to give high-resolution mass spectra (HRMS). All reagents were commercially available and used without further purification, unless otherwise noted. Flash column chromatography was carried out on silica gel (silica gel (40−63 μm)). The combustion analyses were carried out in the microanalytical laboratory of this department.

O-(Hydroxy(((2R,3S)-2-((oleoyloxy)methyl)tetrahydro-2H-pyran-3-yl)oxy)phosphoryl)-L-serine (1). Compound **43a** (111.2 mg, 0.1433 mmol) was dissolved in trifluoroacetic acid at 0 °C and stirred at 0 °C for 30 min. After 30 min, solvent was evaporated under vacuum and the residue was purified by column chromatography $CHCl_3$: MeOH: AcOH = 8:1:1) to yield the compound which had one *tert*-butyl group in its molecule (103.1 mg). The obtained compound was dissolved in trifluoroacetic acid at 0 °C and stirred at room temperature for 2.5 h. After 2.5 h, solvent was evaporated under vacuum and the residue was purified by column chromatography ($CHCl_3$: MeOH: AcOH = 8:1:1 to 6:1:3) to yield **1** (75.9 mg, 0.1347 mmol, 94%, white solid).

^{1}H NMR ($CDCl_3$, 400 MHz): 5.398−5.306 (1H, m), 5.104−5.042 (1H, m), 4.605 (2H, brs), 4.493 (1H, brs), 4.397−4.396 (1H, m), 4.272 (1H, m), 4.120−4.095 (2H, m), 3.602−3.485 (2H, m), 2.390−2.287 (3H, m), 2.035−1.966 (2H, m), 1.794−1.573 (7H, m), 1.261 (20H, m), 0.890−0.856 (3H, m). ^{31}P NMR ($CDCl_3$, 161 MHz): −3.10. HRMS (ESI-TOF: $[M\text{-}H]^-$): Calcd for $C_{27}H_{49}NO_9P^-$: 562.3150, Found 562.3178. Anal. Calcd for $C_{27}H_{50}NO_9P + 0.8CF_3CO_2H$; C, 52.46; H, 7.77; N, 2.14; Found C, 52.20; H, 7.72; N, 2.16. Mp. 122−130 °C.

O-(Hydroxy(((2R,3S)-2-(((3-(2-(undecyloxy)phenyl)propanoyl)oxy)methyl)tetrahydro-2H-pyran-3-yl)oxy)phosphoryl)-L-serine (2). Compound **44a** was dissolved in trifluoroacetic acid at 0 °C. This was stirred at 0 °C for 10 min. After 10 min, the reaction mixture was stirred at room temperature for 3 h. After 3 h, solvent was removed and the residue was purified by column chromatography ($CHCl_3$: MeOH: AcOH = 8:1:1 to 6:1:2) to yield **2** (43.6 mg, 0.0725 mmol, 94%, white soild).

^{1}H NMR ($CDCl_3$, 400 MHz): 7.227−7.171 (1H, m), 7.120−7.057 (1H, m), 6.877−6.805 (m, 2H), 4.597 (brs, 2H), 4.450−4.399 (m, 2H), 4.215−4.070 (3H, m), 3.979 (2H, t, J = 6.8 Hz), 3.575−3.454 (2H, m), 2.939−2.903 (2H, m), 2.751−2.733 (2H, m), 2.299−2.290 (1H, m), 1.816−1.650 (5H, m), 1.427−1.271 (20H, m), 0.878 (3H, t, J = 6.8 Hz). ^{31}P NMR ($CDCl_3$, 161 MHz): −2.90. HRMS (ESI-TOF: $[M\text{-}H]^-$): Calcd for $C_{29}H_{47}NO_{10}P^-$: 600.2943. Found 600.2934. Anal. Calcd for $C_{29}H_{48}NO_{10}P + CF_3CO_2H$; C, 52.02; H, 6.90; N, 1.96. Found C, 52.06; H, 7.01; N, 1.91. Mp. 107−128 °C.

O-(Hydroxy(((2R,3S)-2-(((3-(2-((3-phenoxybenzyl)oxy)phenyl)propanoyl)oxy)methyl)tetrahydro-2H-pyran-3-yl)oxy)phosphoryl)-L-serine (3). Compound **45a** was dissolved in TFA at 0 °Ç and stirred at 0 °C for 10 min. After 10 min, the reaction mixture was stirred at room temperature for 2.5 h. After 2.5 h, the reaction mixture was evaporated and the residue was purified by column chromatography ($CHCl_3$: MeOH: AcOH = 8:1:1 to 6:1:3) to yield **3** (47.3 mg, 0.0751 mmol, 90%, white solid, 71% (2 steps)).

^{1}H NMR ($CDCl_3$, 400 MHz): 7.359−7.314 (3H, m), 7.203−6.879 (10H, m), 5.078 (2H, s), 4.264−4.598 (2H, m), 4.465−4.378 (2H, m), 4.209−4.064 (3H, m), 3.565−3.443 (2H, m), 2.927−2.936 (2H, m), 2.748−2.729 (2H, m), 2.308 (1H, brs), 1.781−1.694 (3H, m). ^{31}P NMR ($CDCl_3$, 161 MHz): −2.73. HRMS (ESI-TOF: $[M\text{-}H]^-$): Calcd for $C_{31}H_{35}NO_{11}P^-$: 628.1953. Found 628.1968. Anal. Calcd for $C_{31}H_{36}NO_{11}P + CF_3CO_2H$; C, 50.17; H, 4.58; N, 1.70. Found C, 50.07; H, 4.95; N, 1.60. Mp. 88−98 °C.

O-(Hydroxy(((2R,3R)-2-((oleoyloxy)methyl)tetrahydro-2H-pyran-3-yl)oxy)phosphoryl)-L-serine (4). Compound **43b** (113.6 mg, 0.1464 mmol) was dissolved in trifluoroacetic acid (2 mL) at 0 °C and stirred at room temperature for 2 h. After 2 h, solvent was removed under vacuum and the residue was purified by column chromatography ($CHCl_3$: MeOH: AcOH = 8:1:1 to 6:1:3) to yield **4** (62.4 mg, 0.1107 mmol, 76%, white solid).

^{1}H NMR ($CDCl_3$, 400 MHz): 5.398−5.255 (1H, m), 5.102−5.042 (1H, m), 4.637 (2H, brs), 4.510 (2H, brs), 4.333−4.312 (1H, m), 4.189−4.161 (2H, m), 3.885−3.871 (1H, m), 3.677−3.619 (1H, m), 2.389−2.352 (2H, m), 2.177−2.149 (1H, m), 2.059−1.969 (3H, m), 1.824−1.793 (1H, m), 1.646−1.573 (5H, m), 1.262 (20H, brs), 0.891−0.857 (3H, m). ^{31}P NMR ($CDCl_3$, 161 MHz): −2.87. HRMS (ESI-TOF: $[M\text{-}H]^-$): Calcd for $C_{27}H_{49}NO_9P^-$: 562.3150. Found 562.3154. Anal. Calcd for $C_{27}H_{50}NO_9P + CF_3COOH$; C, 51.40; H, 7.59; N, 2.07. Found C, 51.00; H, 7.47; N, 2.02. Mp. 121−146 °C.

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*O-(Hydroxy(((2R,3R)-2-(((3-(2-(undecyloxy)phenyl)propanoyl)oxy)methyl)tetrahydro-2H-pyran-3-yl)oxy)phosphoryl)-L-serine (**5**).* Compound **44b** (126.7 mg, 0.1557 mmol) was dissolved in trifluoroacetic acid (2 mL) at 0 °C and stirred at room temperature for 3 h. After 3 h, solvent was removed under vacuum and the residue was purified by column chromatography ($CHCl_3$: MeOH: AcOH = 8:1:1 to 6:1:3) to yield **5** (80.38 mg, 0.1336 mmol, 86%, white solid).

^{1}H NMR ($CDCl_3$, 400 MHz): 7.230−7.171 (1H, m), 7.070−7.053 (1H, m), 6.873−6.799 (2H, m), 4.607−4.583 (2H, m), 4.451 (2H, brs), 4.334−4.285 (1H, m), 4.172−4.131 (2H, m), 3.980 (2H, t, J = 6.8 Hz), 3.767−3.751 (1H, m), 3.625−3.567 (1H, m), 2.933−2.900 (2H, m), 2.748−2.712 (2H, m), 2.163−2.133 (1H, m), 2.010−1.981 (1H, m), 1.827−1.757 (3H, m), 1.537−1.409 (3H, m), 1.365−1.278 (15H, m), 0.885 (3H, t, J = 6.8 Hz). ^{31}P NMR ($CDCl_3$, 161 MHz): −2.96. HRMS (ESI-TOF: [M-H]$^-$): Calcd for $C_{29}H_{47}NO_{10}P^-$: 600.2943. Found 600.2925. Anal. Calcd for $C_{29}H_{48}NO_{10}P$ + 0.7CF_3CO_2H; C, 53.38; H, 7.15; N, 2.06. Found C, 53.75; H, 7.30; N, 2.08. Mp. 160−168 °C.

*O-(Hydroxy(((2R,3R)-2-(((3-(2-((3-phenoxybenzyl)oxy)phenyl)propanoyl)oxy)methyl)tetrahydro-2H-pyran-3-yl)oxy)phosphoryl)-L-serine (**6**).* Compound **45b** (74.0 mg, 0.0879 mmol) was dissolved in trifluoroacetic acid (2 mL) at 0 °C and stirred at room temperature for 3.5 h. After 3.5 h, solvent was removed under vacuum and the residue was purified by column chromatography ($CHCl_3$: MeOH: AcOH = 8:1:1 to 6:1:3) to yield **6** (35.25 mg, 0.0560 mmol, 64%, white solid).

^{1}H NMR ($CDCl_3$, 400 MHz): 7.364−7.325 (3H, m), 7.214−7.085 (5H, m), 7.024−7.005 (2H, m), 6.970−6.945 (1H, m), 6.909−6.889 (2H, m), 5.082 (2H, s), 4.665 (2H, brs), 4.510−4.370 (2H, m), 4.306 (brs, 1H), 4.147−4.122 (m, 2H), 3.758 (brs, 1H), 3.602−3.550 (m, 1H), 2.958 (brs, 2H), 2.744−2.727 (2H, m), 2.199−2.156 (1H, m), 1.994−1.982 (1H, m), 1.733 (1H, brs), 1.534−1.509 (1H, m). ^{31}P NMR ($CDCl_3$, 161 MHz): 0.97. HRMS (ESI-TOF: [M-H]$^-$): Calcd for $C_{31}H_{35}NO_{11}P^-$: 628.1953, Found 628.1934. Anal. Calcd for $C_{31}H_{36}NO_{11}P$ + 1.2CF_3CO_2H; C, 52.34; H, 4.89; N, 1.83. Found C, 52.28; H, 4.99; N, 1.71. Mp. 107−127 °C.

*O-((((2R,3R)-2-(((3-(2-((3-(4-Chlorophenoxy)benzyl)oxy)phenyl)propanoyl)oxy)methyl)tetrahydro-2H-pyran-3-yl)oxy) (hydroxy)-phosphoryl)-L-serine (**13**).* Compound **45c** (18.3 mg, 0.0209 mmol) was dissolved in TFA (1.5 mL) at 0 °C and stirred at 0 °C for 10 min and at room temperature for 1 h. The reaction mixture was evaporated and the residue was purified by column chromatography ($CHCl_3$:MeOH:H_2O= 65:25:4 (190 mL)) to yield **13** (17.4 mg, 0.0262 mmol, quantitative, TFA salt).

^{1}H NMR ($CDCl_3$): 7.257−7.218 (1H, m), 7.185−7.144 (2H, m), 7.100−7.043 (2H, m), 6.985−6.931 (2H, m), 6.851−6.772 (5H, m), 4.976 (2H, s), 4.581 (2H, m), 4.433 (1H, m), 4.348 (1H, m), 4.186 (1H, m), 4.064−4.040 (2H, m), 3.666 (1H, m), 3.524−3.466 (1H, m), 2.845 (2H, m), 2.640−2.606 (2H, m), 2.034 (1H, m), 1.913−1.889 (1H, m), 1.653 (1H, m), 1.468−1.383 (1H, m). ^{31}P NMR ($CDCl_3$): −2.81. HRMS (ESI-TOF [M-H]$^-$): Calcd for $C_{31}H_{34}ClNO_{11}P^-$ 662.1563. Found 662.1576. Anal. Calcd for $C_{31}H_{35}ClNO_{11}P$ + 3.5CF_3CO_2H; C, 42.94 H, 3.63; N, 1.32. Found C, 42.74; H, 3.88 ; N, 1.57. Mp. 171−182 °C.

*O-(Hydroxy(((2R,3R)-2-(((3-(2-((3-(p-tolyloxy)benzyl)oxy)phenyl)-propanoyl)oxy)-methyl)tetrahydro-2H-pyran-3-yl)oxy)phosphoryl)-L-serine (**14**).* Compound **45d** 18.3 mg, 0.0209 mmol) was dissolved in TFA (1.5 mL) at 0 °C and stirred at 0 °C for 10 min and at room temperature for 1 h. The reaction mixture was evaporated and the residue was purified by column chromatography ($CHCl_3$:MeOH:H_2O= 65:25:4 (180 mL)) to yield **14** (16.4 mg, 0.0255 mmol, 75%).

^{1}H NMR ($CDCl_3$, 400 MHz): 7.320 (t, 1H, J = 8.0 Hz), 7.218−7.064 (m, 6H), 6.931−6.898 (m, 5H), 5.077 (s, 2H), 4.752−4.622 (m, 2H), 4.609−4.457 (m, 2H), 4.297−4.291 (m, 1H), 4.165−4.141 (m, 2H), 3.772−3.708 (m, 1H), 3.623−3.598 (m, 1H), 2.964−2.957 (m, 2H), 2.754−2.737 (m, 2H), 2.336 (s, 3H), 2.177−2.152 (m, 1H), 2.076−2.014 (m, 1H), 1.756−1.741 (m, 1H), 1.584−1.527 (m, 1H). ^{31}P NMR ($CDCl_3$, 161 MHz): −2.77. HRMS (ESI-TOF [M-H]$^-$): Calcd for $C_{32}H_{37}NO_{11}P^-$ 642.2110. Found 642.2126. Anal. Calcd for $C_{32}H_{38}NO_{11}P$ + 3.4CF_3CO_2H; C, 45.18; H, 4.02; N, 1.36. Found C, 45.01; H, 4.27; N, 1.57. Mp. 170−178 °C.

*O-((((2R,3R)-3-((3-(2-((3-(4-Chlorophenoxy)benzyl)oxy)phenyl)-propanoyl)oxy)tetrahydro-2H-pyran-2-yl)methoxy)(hydroxy)-phosphoryl)-L-serine (**15**).* Compound **66c** (37.2 mg, 0.0426 mmol) was dissolved in TFA (1 mL) at 0 °C and stirred at 0 °C for 10 min and at room temperature for 1 h. The reaction mixture was evaporated and the residue was purified by column chromatography ($CHCl_3$:MeOH:H_2O= 65:25:4 (360 mL)) to yield **15** (36.7 mg, 0.0553 mmol, 100%).

^{1}H NMR ($CDCl_3$, 400 MHz): 7.361 (t, 1H, J = 8.0 Hz), 7.323−7.293 (m, 2H), 7.225−7.173 (m, 2H), 7.106−7.089 (m, 1H), 7.052 (m, 1H), 6.917−6.899 (m, 5H), 5.109 (s, 2H), 5.031(m, 1H), 4.558 (m, 2H), 4.468 (m, 1H), 4.144−4.117 (m, 1H), 3.981−3.932 (m, 2H), 3.819 (m, 1H), 3.634−3.582 (m, 1H), 3.060−2.945 (m, 2H), 2.847−2.698 (m, 2H), 1.835−1.689 (m, 3H), 1.470−1.422 (m, 1H). ^{31}P NMR ($CDCl_3$, 400 MHz): −2.69. HRMS (ESI-TOF [M-H]$^-$): Calcd for $C_{31}H_{34}ClNO_{11}P^-$ 662.1564, Found 662.1589. Anal. Calcd for $C_{31}H_{35}ClNO_{11}P$ + 6CF_3CO_2H + 2H_2O; C, 37.31; H, 3.28 ; N, 1.01; Found C, 37.14; H, 3.42; N, 1.30. Mp. 178−189 °C.

*O-(Hydroxy(((2R,3R)-3-((3-(2-((3-(p-tolyloxy)benzyl)oxy)phenyl)-propanoyl)oxy)tetrahydro-2H-pyran-2-yl)methoxy)phosphoryl)-L-serine (**16**).* Compound **66d** (14.9 mg, 0.0175 mmol) was dissolved in TFA (1 mL) at 0 °C and stirred at 0 °C for 10 min and at room temperature for 1.5 h. The reaction mixture was evaporated and the residue was purified by column chromatography ($CHCl_3$:MeOH:H_2O= 65:25:4(360 mL)) to yield **16** (46.9 mg, 0.0729 mmol, quantitative).

^{1}H NMR ($CDCl_3$, 400 MHz): 7.319−7.280 (m, 1H), 7.199−7.030 (m, 6H), 6.908−6.869 (m, 5H), 5.062 (s, 2H), 4.991(m, 1H), 4.521 (m, 2H), 4.447−4.412 (m, 1H), 4.096−4.071 (m, 1H), 3.979−3.900 (m, 2H), 3.779 (m, 1H), 3.592−3.540 (m, 1H), 3.073−2.917 (m, 2H), 2.873−2.723 (m, 2H), 2.329 (s, 3H), 1.802−1.652 (m, 3H), 1.432−1.406 (m, 1H). ^{31}P NMR ($CDCl_3$, 161 MHz): δ −2.58. HRMS (ESI-TOF [M-H]$^-$): Calcd for $C_{32}H_{37}NO_{11}P^-$ 642.2110. Found 642.2121. Anal. Calcd for $C_{32}H_{38}NO_{11}P$ + 6CF_3CO_2H; C, 39.80; H, 3.34; N, 1.05. Found C, 39.72; H, 3.66; N, 1.06. Mp. 179−189 °C.

*O-(Hydroxy(((2R,3S)-2-((oleoyloxy)methyl)-3,6-dihydro-2H-pyran-3-yl)oxy)phosphoryl)-L-serine (**21**).* Compound **50a** (98.8 mg, 0.1277 mmol) was dissolved in trifluoroacetic acid (2 mL) at 0 °C, stirred at 0 °C for 10 min and stirred at room temperature for 2 h. After 2 h, the reaction mixture was evaporated to remove solvent and the residue was purified by column chromatography ($CHCl_3$: MeOH: AcOH = 7:1:1 to 6:1:4) to yield **21** (34.6 mg, 0.0616 mmol, 48%, white solid).

^{1}H NMR ($CDCl_3$, 400 MHz): 5.900−5.875 (1H, m), 5.803−5.718 (1H, m), 5.295−5.211 (1H, m), 5.003−4.942 (m, 1H), 4.570 (brs, 1H), 4.470−4.302 (m, 3H), 4.183−4.142 (m, 3H), 3.697−3.621 (1H, m), 2.308−2.271 (2H, m), 1.928−1.878 (2H, m), 1.552−1.496 (4H, m), 1.174 (19H, brs), 0.806−0.772 (3H, m). ^{31}P NMR ($CDCl_3$): = −2.72. HRMS (ESI-TOF: [M-H]$^-$): Calcd for $C_{27}H_{47}NO_9P^-$: 560.2994, Found 560.3027. Anal. Calcd for $C_{27}H_{48}NO_9P$ + CF_3CO_2H + H_2O; C, 48.84; H, 7.10; N, 1.92; Found C, 48.93; H, 7.28; N, 2.13. Mp. 140−151 °C.

*O-(Hydroxy(((2R,3S)-2-(((3-(2-(undecyloxy)phenyl)propanoyl)-oxy)methyl)-3,6-dihydro-2H-pyran-3-yl)oxy)phosphoryl)-L-serine (**22**).* Compound **51a** (35.5 mg, 0.0437 mmol) was dissolved in trifluoroacetic acid (2 mL) at 0 °C and stirred at room temperature for 1.5 h. After 1.5 h, solvent was removed under vacuum and the residue was purified by column chromatography ($CHCl_3$: MeOH: AcOH = 6:1:2 to 4:1:3) to yield **22** (15.3 mg, 0.0255 mmol, 58%, white solid, (21%, 2 steps)).

^{1}H NMR ($CDCl_3$, 400 MHz): 7.214−7.176 (1H, m), 7.076−7.059 (1H, m), 6.895−6.829 (2H, m), 6.027−6.003 (1H, m), 5.876−5.809 (1H, m), 4.738−4.424 (5H, m), 4.310−4.254 (3H, m), 4.011−3.977 (2H, m), 3.864−3.861 (1H, m), 2.951−2.866 (2H, m), 2.773−2.756 (2H, m), 1.816−1.746 (2H, m), 1.427−1.267 (16H, m), 0.890−0.857 (3H, m). ^{31}P NMR ($CDCl_3$, 161 MHz): −1.85. HRMS (ESI-TOF [M-H]$^-$): Calcd for $C_{29}H_{45}NO_{10}P^-$: 598.2787. Found 598.2779. Anal.

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Calcd for $C_{29}H_{46}NO_{10}P$ + 1.5CF_3CO_2H; C, 49.87; H, 6.17; N, 1.82. Found C, 49.72; H, 6.10; N, 1.68. Mp.141−155 °C.

*O-(Hydroxy(((2R,3S)-2-(((3-(2-((3-phenoxybenzyl)oxy)phenyl)-propanoyl)oxy)methyl)-3,6-dihydro-2H-pyran-3-yl)oxy)-phosphoryl)-L-serine (**23**).* Compound **52a** (98.0 mg, 0.1167 mmol) was dissolved in trifluoroacetic acid (2 mL) at 0 °C and stirred at room temperature for 1 h. After 1 h, solvent was removed under vacuum and the residue was purified by column chromatography ($CHCl_3$: MeOH: AcOH = 6:1:2 to 4:1:3) to yield **23** (12.9 mg, 0.0206 mmol, 18%, white solid (11%, 2 steps)).

^{1}H NMR ($CDCl_3$, 400 MHz): 7.364−7.302 (3H, m), 7.209−7.7.026 (5H, m), 7.009−6.863 (5H, m), 6.027−5.944 (m, 1H), 5.801−5.775 (m, 1H), 5.063 (s, 2H), 4.666−4.102 (m, 8H), 3.774−3.757 (1H, m), 2.988−2.928 (2H, m), 2.747−2.666 (2H, m). ^{31}P NMR ($CDCl_3$, 161 MHz): −2.66. HRMS (ESI-TOF [M-H]$^-$): Calcd for $C_{31}H_{33}NO_{11}P^-$ 626.1797. Found 626.1811. Anal. Calcd for $C_{31}H_{34}NO_{11}P$ + 2CF_3CO_2H + H_2O; C, 48.12; H, 4.38; N, 1.60. Found C, 48.15; H, 4.26; N, 1.78. Mp. 134−150 °C.

*O-(Hydroxy(((2R,3R)-2-((oleoyloxy)methyl)-3,6-dihydro-2H-pyran-3-yl)oxy)phosphoryl)-L-serine (**24**).* Compoud **50b** (114.8 mg, 0.1483 mmol) was dissolved in trifluoroacetic acid (2 mL) at 0 °C, stirred at 0 °C for 10 min and stirred at room temperature for 2.5 h. After 2.5 h, the reaction mixture was evaporated to remove solvent and the residue was purified by column chromatography ($CHCl_3$: MeOH: AcOH = 7:1:1 to 6:1:4) to yield **24** (74.8 mg, 0.1332 mmol, 90%, white solid).

^{1}H NMR ($CDCl_3$, 400 MHz): 6.151−5.930 (2H, m), 5.396−5.306 (1H, m), 5.102−5.041 (1H, m), 4.644−4.255 (5H, m), 4.297−4.255 (2H, m), 3.987 (1H, brs), 2.434−2.371 (2H, m), 2.061−1.970 (2H, m), 1.647−1.529 (4H, m), 1.264 (21H, brs), 0.892−0.858 (3H, m). ^{31}P NMR ($CDCl_3$, 161 MHz): −2.66. HRMS (ESI-TOF: [M-H]$^-$): Calcd for $C_{27}H_{47}NO_9P^-$: 560.2994. Found 560.2991. Anal. Calcd for $C_{27}H_{48}NO_9P$ + 0.9CF_3CO_2H + H_2O; C, 50.70; H, 7.52; N, 2.05. Found C, 50.95; H, 7.33; N, 2.01. Mp. 130−140 °C.

*O-(Hydroxy(((2R,3R)-2-(((3-(2-(undecyloxy)phenyl)propanoyl)-oxy)methyl)-3,6-dihydro-2H-pyran-3-yl)oxy)phosphoryl)-L-serine (**25**).* Compound **51b** (91.1 mg, 0.1122 mmol) was dissolved in trifluoroacetic acid (2 mL) at 0 °C and stirred at room temperature for 2.5 h. After 2.5 h, solvent was removed under vacuum and the residue was purified by column chromatography ($CHCl_3$: MeOH: AcOH = 6:1:2 to 5:1:3) to yield **25** (52.1 mg, 0.0869 mmol, 77%, white solid).

^{1}H NMR ($CDCl_3$, 400 MHz): 7.201−7.057 (2H, m), 6.866−6.829 (2H, m), 6.126−6.018 (2H, m), 4.603−4.189 (m, 8H), 3.976 (t, 2H, *J* = 6.8 Hz), 3.877 (m, 1H), 2.940−2.907 (m, 2H), 2.759−2.723 (2H, m), 1.824−1.754 (2H, m), 1.455−1.271 (16H, m), 0.897−0.863 (3H, m). ^{31}P NMR ($CDCl_3$, 161 MHz): −2.64. HRMS (ESI-TOF [M-H]$^-$): Calcd for $C_{29}H_{45}NO_{10}P^-$ 598.2787. Found 598.2785. Anal. Calcd for $C_{29}H_{46}NO_{10}P$ + 0.8CF_3CO_2H; C, 53.20; H, 6.78; N, 2.03. Found C, 53.47; H, 6.84; N, 2.12. Mp. 151−163 °C.

*O-(Hydroxy(((2R,3R)-2-(((3-(2-((3-phenoxybenzyl)oxy)phenyl)-propanoyl)oxy)methyl)-3,6-dihydro-2H-pyran-3-yl)oxy)-phosphoryl)-L-serine (**26**).* Compound **52b** (43.1 mg, 0.0513 mmol) was dissolved in trifluoroacetic acid (1 mL) at 0 °C and stirred at room temperature for 2.5 h. After 2.5 h, solvent was removed under vacuum and the residue was purified by column chromatography ($CHCl_3$: MeOH: AcOH = 6:1:2 to 5:1:3) to yield **26** (13.7 mg, 0.0128 mmol, 43%, white solid (8%, 2 steps)).

^{1}H NMR ($CDCl_3$, 400 MHz): 7.361−7.311 (3H, m), 7.209−7.078 (5H, m), 7.015−6.871 (5H, m), 6.114−6.090 (1H, m), 5.980 (1H, m), 5.078 (2H, s), 4.571−4.502 (3H, m), 4.466−4.434 (1H, m), 4.386−4.337 (2H, m), 4.260−4.165 (2H, m), 3.858−3.842 (1H, m), 3.016−2.901 (2H, m), 2.811−2.722 (2H, m). ^{31}P NMR ($CDCl_3$, 161 MHz): −3.09. HRMS (ESI-TOF [M-H]$^-$): Calcd for $C_{31}H_{33}NO_{11}P^-$: 626.1797. Found 626.1801. Anal. Calcd for $C_{31}H_{34}NO_{11}P$ + 2CF_3CO_2H; C, 49.13; H, 4.24; N, 1.64. Found C, 48.78; H, 4.24; N, 1.61. Mp. 175−188 °C.

*O-(Hydroxy(2-(((3-(2-(undecyloxy)phenyl)propanoyl)oxy)-methyl)phenoxy)phosphoryl)-L-serine (**33**).* Compound **57a** (127.0 mg, 0.1576 mmol) was dissolved in TFA (2 mL) at 0 °C and stirred at 0 °C for 10 min and at room temperature for 1.5 h. The reaction mixture was evaporated and the residue was purified by column chromatography ($CHCl_3$: MeOH: AcOH = 6:1:2 (270 mL) to 6:1:3 (300 mL) to yield **33** (93.3 mg, 0.1572 mmol, 100%, white solid).

^{1}H NMR ($CDCl_3$, 400 MHz): 7.309−7.254 (m, 2H), 7.226−7.120 (m, 3H), 7.024−7.005 (m, 1H), 6.892−6.824 (m, 2H), 5.227 (s, 2H), 4.690 (m, 2H), 4.494 (m, 1H), 3.991 (t, 2H, *J* = 6.8 Hz), 2.972−2.935 (m, 2H), 2.796−2.759 (m, 2H), 1.820−1.749 (m, 2H), 1.456−1.402 (m, 2H), 1.322−1.253 (m, 14H), 0.880 (t, 3H, *J* = 6.8 Hz). ^{31}P NMR ($CDCl_3$, 161 MHz): −6.99.

HRMS (ESI-TOF [M-H]$^-$): Calcd for $C_{30}H_{43}NO_9P^-$ 592.2681. Found 592.2664. Anal. Calcd for $C_{30}H_{44}NO_9P$ + 1.1CF_3CO_2H; C, 53.76; H, 6.27; N, 1.95. Found C, 53.84; H, 6.29; N, 1.99. Mp. 145−156 °C.

*O-(Hydroxy(3-(((3-(2-(undecyloxy)phenyl)propanoyl)oxy)-methyl)phenoxy)phosphoryl)-L-serine (**34**).* Compound **57b** (23.5 mg, 0.0292 mmol) was dissolved in TFA (1.3 mL) at 0 °C and stirred at 0 °C for 10 min and at room temperature for 1.5 h. The reaction mixture was evaporated and the residue was purified by column chromatography ($CHCl_3$: MeOH: H_2O= 65:25:4 (190 mL)) to yield **34** (16.2 mg, 0.0273 mol, 94%).

^{1}H NMR ($CDCl_3$, 400 MHz): 7.323−7.284 (m, 1H), 7.201−7.158 (m, 1H), 7.129−7.110 (m, 1H), 7.051−7.026 (m, 2H), 7.004−6.983 (m, 1H), 6.870−6.807 (m, 2H), 5.085 (s, 2H), 4.643 (m, 2H), 4.431 (m, 1H), 3.994−3.961 (m, 2H), 2.948−2.911 (m, 2H), 2.755−2.718 (m, 2H), 1.812−1.742 (m, 2H), 1.467−1.398 (m, 2H), 1.353−1.249 (m, 14H), 0.889−0.854 (m, 3H).

^{31}P NMR ($CDCl_3$, 161 MHz): −7.26. HRMS (ESI-TOF [M-H]$^-$): Calcd for $C_{30}H_{43}NO_9P^-$ 592.2681. Found 592.2680. Anal. Calcd for $C_{30}H_{44}NO_9P$ + 1.5CF_3CO_2H; C, 51.83; H, 5.96; N, 1.83. Found C, 52.04; H, 6.09; N, 1.95. Mp. 181−190 °C.

*O-(Hydroxy(4-(((3-(2-(undecyloxy)phenyl)propanoyl)oxy)-methyl)phenoxy)phosphoryl)-L-serine (**35**).* Compound **57c** (43.3 mg, 0.0537 mmol) was dissolved in TFA (1.5 mL) at 0 °C and stirred at 0 °C for 10 min and at room temperature for 2 h. The reaction mixture was evaporated and the residue was purified by column chromatography ($CHCl_3$: MeOH: H_2O= 65:25:4) to yield **35** (36.6 mg, 0.0607 mmol, quatitative, TFA salt).

^{1}H NMR ($CDCl_3$, 400 MHz): 7.263−7.254 (2H, m), 7.210−7.171 (1H, m), 7.044−7.026 (3H, m), 6.886−6.831 (2H, m), 5.110 (2H, s), 4.721 (2H, m), 4.531 (1H, m), 3.991 (2H, t, *J* = 6.4 Hz), 2.955 (2H, t, *J* = 7.6 Hz), 2.762 (2H, t, *J* = 7.6 Hz), 1.822−1.752 (2H, m), 1.459−1.405 (2H, m), 1.332−1.267 (14H, m), 0.871 (3H, t, *J* = 6.8 Hz). ^{31}P NMR ($CDCl_3$, 400 MHz): −6.77. HRMS (ESI-TOF [M-H]$^-$): Calcd for $C_{30}H_{43}NO_9P^-$: 592.2681. Found 592.2680. Anal. Calcd for $C_{30}H_{44}NO_9P$ + 2CF_3CO_2H + H_2O; C, 48.63; H, 5.76; N, 1.67. Found C, 48.55; H, 5.86; N, 1.88. Mp. 184−193 °C.

*((2R,3S)-3-Acetoxy-3,6-dihydro-2H-pyran-2-yl)methyl acetate (**36a**).* Tri-*O*-acetyl-D-glucal (1.0003 g, 3.6731 mmol) and triethylsilane (512.53 mg, 4.41 mmol) were dissolved in CH_2Cl_2 (5 mL) at room temperature under Ar. The solution was cooled to 0 °C. BF_3·OEt_2 (521.32 mg, 3.67 mmol) was added dropwise to the solution above at 0 °C under Ar atmosphere. The reaction mixture was stirred 0 °C under Ar for 15 min. The reaction mixture was quenched with 10% aqueous $NaHCO_3$ solution (0.5 mL) and diluted with ether (5.5 mL). The whole was washed with H_2O (5 mLx2), brine (5 mL), and then dried over $MgSO_4$. Combined organic layer was evaporated to yield **36a** (784.6 mg, 3.66 mmol, 100%, colorless oil). ^{1}H NMR ($CDCl_3$, 400 MHz): 5.866−5.834 (1H, m), 5.666 (1H, ddd, *J* = 10.4 Hz, 4.4 Hz, 2.4 Hz), 5.177−5.133 (1H, m), 4.126−4.054 (4H, m), 3.628 (1H, sp, *J* = 8.4 Hz, 2.8 Hz), 1.997 (3H, s), 1.981 (3H, s). ^{13}C NMR ($CDCl_3$, 100 MHz): 170.68, 170.16, 129.42, 124.18, 73.76, 65.19, 65.00, 63.19, 20.91, 20.70. HRMS (ESI-TOF [M + Na]$^+$): Calcd for $C_{10}H_{14}NaO_5^+$: 237.0733. Found: 237.0731. Anal. Calcd for $C_{10}H_{14}O_5$ + 0.2H_2O: C, 55.14; H, 6.66; N, 0.00. Found: C, 55.17; H, 6.45; N, 0.00.

*((2R,3R)-3-Acetoxy-3,6-dihydro-2H-pyran-2-yl)methyl acetate (**36b**).* Tri-*O*-acetyl-O-galactal (5.2392 mg, 19.2441 mmol) was dissolved in CH_2Cl_2 (100 mL). Triethylsilane (2.6852 g, 23.0929 mmol) was added to the solution above at 0 °C. BF_3·OEt_2 (3.2776 g, 23.0929 mmol) was added dropwise to the solution above at 0 °C. The reaction mixture was stirred at room temperature under Ar for 15

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min. Saturated aqueous $NaHCO_3$ solution (50 mL) was added to the reaction mixture and the whole was extracted with CH_2Cl_2 (20 mLx3). Combined organic layer was washed with water, brine, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography (ether: petroleum ether =1:1) to yield **36b** (3.9502 mg, 18.3499 mmol, 96%, colorless oil).

^{1}H NMR ($CDCl_3$, 400 MHz): 6.092−6.052 (1H, m), 5.996−5.947 (1H, m), 5.084−5.059 (1H, tt, J = 0.4 Hz, 2.4 Hz), 4.304 (1H, dddd, J = 3.6 Hz, 2.0 Hz), 4.224−4.142 (3H, m), 3.850 (1H, sp, J = 2.4 Hz), 2.054 (3H, s), 2.047 (3H, s). ^{13}C NMR ($CDCl_3$, 100 MHz): 170.73, 170.47, 132.33, 122.09, 73.72, 65.70, 64.26, 63.30, 20.89, 20.80. HRMS (ESI-TOF [M + Na]$^+$): Calcd for $C_{10}H_{14}NaO_5^+$: 237.0733. Found 237.0732. Anal. Calcd for $C_{10}H_{14}O_5$; C, 56.07; H, 6.59; N, 0.00. Found: C, 55.90; H, 6.40; N, 0.00.

*(2R,3S)-2-(Acetoxymethyl)tetrahydro-2H-pyran-3-yl acetate (**37a**).* Compound **36a** (2.1379 g, 9.9799 mmol) was dissolved in MeOH (24 mL) and 10% Pd/C (220 mg) in MeOH (6 mL) was added to the solution above. The reaction mixture was placed under H_2 atmosphere and stirred at room temperature for 3 h. After 3 h, the reaction mixture was filtered on Celite and the filtrate was evaporated under vacuum to yield **37a** (2.0224 mg, 9.3530 mmol, 94%, colorless oil).

^{1}H NMR ($CDCl_3$, 400 MHz): 4.676−4.613 (1H, m), 4.186−4.093 (1H, m), 3.970−3.926 (1H, m), 3.467−3.424 (1H, m), 3.403−3.338 (1H, dt, J = 2.8 Hz, 11.6 Hz), 2.216−2.177 (1H, m), 2.054 (3H, s), 2.007 (3H, s), 1.760−1.668 (2H, m), 1.489−1.419 (1H, m). ^{13}C NMR ($CDCl_3$, 100 MHz):170.90, 169.91, 77.53, 68.06, 67.90, 63.05, 29.20, 24.83, 21.03, 20.82. HRMS (ESI-TOF [M + Na]$^+$): Calcd for $C_{10}H_{16}NaO_5^+$: 239.0890. Found: 239.0919. Anal. Calcd for $C_{10}H_{16}O_5$ + 0.1CH_2Cl_2: C, 53.98; H, 7.27; N, 0.00. Found: C, 54.14 H, 7.24; N, 0.00.

*(2R,3R)-2-(Acetoxymethyl)tetrahydro-2H-pyran-3-yl acetate (**37b**).* Compound **36b** (555.6 mg, 2.5936 mol) was dissolved in MeOH (7 mL) and 10% Pd/C (56 mg) in MeOH (3 mL) was added to the solution above. The reaction mixture was placed under H_2 atmosphere and stirred at room temperature for 3 h. After 3 h, the reaction mixture was filtered on Celite and the filtrate was evaporated to yield **37b** (539.6 mg, 2.4955 mmol, 96%, colorless oil).

^{1}H NMR ($CDCl_3$, 400 MHz): 4.906 (1H, brs), 4.121−4.019 (3H, m), 3.699−3.664 (1H, m), 3.502 (1H, dt, J = 12.0 Hz, 2.4 Hz), 2.090 (3H, s), 2.048−2.000 (4H, m), 1.956−1.836 (1H, m), 1.734−1.646 (1H, m), 1.456−1.400 (1H, m). ^{13}C NMR ($CDCl_3$, 100 MHz): 170.77, 170.48, 75.76, 68.00, 67.03, 63.90, 27.51, 21.08, 20.84, 20.53. HRMS (ESI-TOF [M + Na]$^+$): Calcd for $C_{10}H_{16}NaO_5^+$: 239.0890. Found 239.0880.

*(2R,3S)-2-(Hydroxymethyl)tetrahydro-2H-pyran-3-ol (**38a**).* Compound **37a** (1.9824 g, 9.1680 mmol) was dissolved in MeOH (22 mL) and sodium methoxide (247.6 mg, 4.5840 mmol) was added to the solution at room temperature. The reaction mixture was stirred at room temperature under Ar for 1.5 h. After 1.5 h, the solvent was removed and the residue was dissolved in $CHCl_3$. This solution was filtered on Celite and the filtrate was evaporated to yield **38a** (1.2031 mg, 9.1034 mmol, 99%, colorless oil).

^{1}H NMR ($CDCl_3$, 400 MHz): 3.931−3.901 (1H, m), 3.853−3.751 (2H, m), 3.584−3.522(1H, m), 3.397−3.332 (1H, m), 3.150−3.105 (1H, m), 2.598 (2H, brs), 2.134−2.083 (1H, m), 1.710−1.643 (2H, m), 1.483−1.381 (1H, m). ^{13}C NMR ($CDCl_3$, 100 MHz): 81.73, 67.65, 67.50, 63.35, 32.51, 25.40. HRMS (ESI-TOF [M + Na]$^+$): Calcd for $C_6H_{12}NaO_3^+$: 155.0679. Found: 155.0677.

*(2R,3R)-2-(Hydroxymethyl)tetrahydro-2H-pyran-3-ol (**38b**).* Compound **37b** (508.9 mg, 2.3535 mmol) was dissolved in MeOH (7.5 mL) and sodium methoxide (63.6 mg, 1.1768 mmol) was added to the solution at room temperature. The reaction mixture was stirred at room temperature under Ar for 1.5 h. After 1.5 h, solvent was removed and the residue was dissolved in $CHCl_3$. This solution was filtered on Celite and the filtrate was evaporated to yield crude **38b** (397.6 mg, 3.0085 mmol, quantitative, yellow oil).

^{1}H NMR ($CDCl_3$, 400 MHz): 4.028−4.008 (1H, m), 3.850−3.762 (3H, m), 3.521−3.465 (1H, m), 3.367 (1H, s), 3.113 (1H, brs), 2.003−1.902 (2H, m), 1.665−1.606 (1H, m), 1.398−1.367 (1H, m). ^{13}C NMR ($CDCl_3$, 100 MHz): 79.11, 68.57, 66.34, 64.08, 30.36, 20.04. HRMS (ESI-TOF [M + Na]$^+$): Calcd for $C_6H_{12}NaO_3^+$ 155.0679. Found 155.0664.

*(2R,3S)-2-(((tert-Butyldimethylsilyl)oxy)methyl)tetrahydro-2H-pyran-3-ol (**39a**).* Compound **38a** (24.0 mg, 0.1816 mmol) and imidazole (27.2 mg, 0.3995 mmol) was dissolved in DMF (0.25 mL) and cooled to 0 °C. *t*-Butyldimethylchloro silane (32.8 mg, 0.2179 mmol) was added to the solution above at 0 °C and the reaction mixture was stirred at room temperature under Ar for 13 h. After 13 h, H_2O (1.5 mL) and diethyl ether (1.5 mL) was added to the reaction mixture and organic layer was separated. This organic layer was washed with H_2O (1.5 mLx2), brine (2 mL) and dried over $MgSO_4$, then evaporated. The residue was purified by column chromatography to yield **39a** (35.5 mg, 0.1441 mmol, 79%, colorless oil).

^{1}H NMR ($CDCl_3$, 400 MHz): 3.922−3.849 (2H, m), 3.692 (1H, dd, J = 10.0, 8.0 Hz)), 3.571 (2H, dddd, J = 10.8 Hz, 8.8 Hz, 4.8 Hz, 2.0 Hz), 3.368−3.303 (1H, m), 3.193−3.139 (1H, m), 2.131−2.075 (1H, m), 1.684−1.616 (2H, m), 1.474−1.371 (1H, m), 0.902 (9H, s), 0.103 (1H, s), 0.097 (3H, s). ^{13}C NMR ($CDCl_3$, 100 MHz): 79.33, 71.14, 67.61, 66.74, 31.59, 25.82, 24.91, 18.16, −5.57, −5.65. HRMS (ESI-TOF [M + Na]$^+$): Calcd For $C_{12}H_{26}NaO_3Si^+$: 269.1543. Found 269.1554. Anal. Calcd for $C_{12}H_{26}O_3Si$ + 0.15CH_2Cl_2; C, 56.31; H, 10.23; N, 0.00. Found: C, 56.50 H, 10.04; N, 0.00.

*(2R,3R)-2-(((tert-Butyldimethylsilyl)oxy)methyl)tetrahydro-2H-pyran-3-ol (**39b**).* Compound **38b** (32.5 mg, 0.2459 mmol) was dissolved in CH_2Cl_2 (0.7 mL) and imidazole (36.8 mg, 0.5410 mmol) was added to the solution. This was cooled to 0 °C and *tert*-butyldimethylsilyl chloride (44.5 mg, 0.2951 mmol) was added. The reaction mixture was stirred at room temperature under Ar for 13 h. After, 13 h, H_2O (5 mL) was added to the reaction mixture and the whole was extracted CH_2Cl_2 (5 mLx3). Combined organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography (*n*-hexane: ethyl acetate =4:1) to yield **39b** (47.0 mg, 0.1907 mmol, 78%, colorless oil). ^{1}H NMR ($CDCl_3$, 400 MHz): 4.028−3.979 (1H, m), 3.910−3.894 (1H, m), 3.822−3.741 (2H, m), 3.504−3.438 (1H, m), 3.336−3.308 (1H, m), 2.774 (1H, brs), 2.056−1.915 (2H, m), 1.664−1.576 (1H, m), 1.395−1.330 (1H, m), 0.894 (9H, s), 0.082 (3H, s), 0.075 (3H, s). ^{13}C NMR ($CDCl_3$, 100 MHz): 79.04, 68.66, 65.71, 64.52, 30.32, 25.89, 20.19, 18.30, −5.43, −5.49. HRMS (ESI-TOF [M + Na]$^+$): Calcd for $C_{12}H_{26}NaO_3Si^+$: 269.1543. Found 269.1554. Anal. Calcd for $C_{12}H_{26}O_3Si$; C, 58.49; H, 10.63; N, 0.00. Found C, 58.19; H, 10.39; N, 0.00.

Phosphoserine unit. **40** was prepared as described previously, starting from *L*-serine.[19]

*tert-Butyl O-(tert-butoxy(diisopropylamino)phosphanyl)-N-(tert-butoxycarbonyl)-L-serinate (**40**).* Bis(diisopropylamino)*tert*-butylphosphine (1.0 g, 3.83 mmol) was dissolved in CH_2Cl_2 (15 mL) and toluene (1.5 mL). *tert*-Butyl (*tert*-butoxycarbonyl)-*L*-serinate (1.301 g, 4.979 mmol) was added to the solution and the solvent was evaporated to remove containing water. The residue was dissolved in dry CH_2Cl_2 (15 mL) under Ar atmosphere and 1*H*-tetrazole in THF (15 mL) was added to the solution at room temperature. The reaction mixture was stirred for 3.5 h. After 3.5 h, the reaction was quenched with saturated aqueous $NaHCO_3$ (20 mL) and the whole was extracted with CH_2Cl_2 (20 mL x 3). Combined organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography (*n*-hexane: ethyl acetate: Et_3N = 35:4:1) to yield **40** (1.56 g, 3.36 mmol, 68%, yellow oil).

^{1}H NMR ($CDCl_3$, 400 MHz): 5.431 (1/2H, d, J = 6.3 Hz), 5.260 (1/2H, d, J = 6.3 Hz), 3.844−3.795 (1H, m), 3.676−3.596 (1H, m), 3.539−3.446 (2H, m), 4.135−4.071 (1H, m), 3.844−3.795 (1H, m), 3.676−596, (1H, m), 3.539−3.446 (2H, m), 1.376−1.368 (9H, m), 1.347−1.340 (9H, m), 1.273−1.237 (9H, m), 1.089−1.052 (12H, m). ^{13}C NMR (CD_2Cl_2, 100 MHz): 168.74, 168.72, 80.56, 80.39, 78.20, 78.14, 74.03, 73.92, 62.85, 62.71, 62.46, 62.32, 54.48, 54.43, 54.35, 42.32, 42.27, 42.20, 42.14, 29.87, 27.33, 27.30, 26.98, 23.70, 23.63, 23.58, 23.52, 23.24, 23.16. ^{31}P NMR (CD_2Cl_2, 161 MHz): 138.92, 138.44. HRMS (ESI-TOF [M + Na]$^+$): Calcd for $C_{22}H_{45}N_2NaO_6P^+$:

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487.2907. Found: 487.2906. Anal. Calcd for $C_{22}H_{45}N_2O_6P$: C, 56.88; H, 9.76; N, 6.03. Found: C, 55.94; H, 9.72; N, 5.86.

*tert-Butyl O-(tert-butoxy(((2R,3S)-2-(((tert-butyldimethylsilyl)-oxy)methyl)tetrahydro-2H-pyran-3-yl)oxy)phosphoryl)-N-(tert-butoxycarbonyl)-L-serinate (**41a**).* **40** (338.0 mg, 0.7486 mmol) was dissolved in CH_2Cl_2 (5 mL) and toluene (0.5 mL) and coevaporated to remove containing water. **39a** (276.7 mg, 1.123 mmol) was added to the solution above and dissolved in CH_2Cl_2 (5 mL) and toluene (0.5 mL) then, coevaporated. The residue was dissolved in dry CH_2Cl_2 (5 mL) and 1H-tetrazole (209.7 mg, 2.994 mmol) in THF (5 mL) was added to the solution at room temperature under Ar. The reaction mixture was stirred at room temperature under Ar for 13 h. TBHP was added to the reaction mixture and stirred at room temperature under Ar for 1.5 h. After 1.5 h, the reaction mixture was quenched with H_2O (10 mL) and extracted with CH_2Cl_2 (5 mLx3). The organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography (*n*-hexane: ethyl acetate =4:1 to 2:1) to yield **41a** (397.1 mg, 0.6345 mmol, 85%, colorless oil (57%, 2 steps)).

^{1}H NMR ($CDCl_3$, 400 MHz): 5.503−5.423 (1H, m), 4.337−4.216 (2H, m), 4.193−4.150 (1H, m), 4.106−4.028 (2H, m), 3.913−3.860 (1H, m), 3.696−3.641 (1H, m), 3.342−3.279 (1H, m), 3.239−3.197 (1H, m), 2.351−2.323 (1H, m), 1.656−1.562 (3H, m), 1.472−1.455 (18H, m), 1.427 (9H, s), 0.876 (9H, s), 0.057−0.045 (6H, m). ^{13}C NMR ($CDCl_3$, 100 MHz): 168.31, 155.20, 83.59, 83.52, 82.60, 82.55, 81.40, 81.30, 79.87, 72.42, 72.36, 67.24, 67.22, 63.40, 54.42, 54.33, 30.90, 30.72, 29.84, 29.79, 28.432, 27.96, 26.00, 25.95, 25.05, 25.01, 18.48, 18.45, −5.10, −5.13. ^{31}P NMR ($CDCl_3$, 161 MHz): −6.379. −6.415. HRMS (ESI-TOF [M + Na]$^+$): Calcd for $C_{28}H_{56}NNaO_{10}PSi^+$: 648.3303. Found 648.3312. Anal. Calcd for $C_{28}H_{56}NO_{10}PSi$; C, 53.74; H, 9.02; N, 2.24. Found C, 53.71; H, 9.01; N, 2.32.

*tert-Butyl O-(tert-butoxy(((2R,3R)-2-(((tert-butyldimethylsilyl)-oxy)methyl)tetrahydro-2H-pyran-3-yl)oxy)phosphoryl)-N-(tert-butoxycarbonyl)-L-serinate (**41b**).* **40** (439.2 mg, 0.9726 mmol) and compound **39b** (359.5 mg, 1.4589 mmol) were dissolved in CH_2Cl_2 (3 mL) and toluene (0.5 mL) and evaporated to remove containing water. The residue was dissolved in dry CH_2Cl_2 (6.5 mL) and *1H*-tetrazole (272.5 mg, 3.8904 mmol) in THF (6.5 mL) was added to the solution. This reaction mixture was stirred at room temperature under Ar for 19 h. After 17 h, *tert*-butylhydroperoxide (0.3890 mg, 1.9452 mmol) was added to the reaction mixture and stirred at room temperature under Ar for 1.5 h. After 1.5 h, the reaction mixture was quenched by water (15 mL) and the whole was extracted with CH_2Cl_2 (10 mLX3). Combined organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography (*n*-hexane: ethyl acetate =4:1 to 2:1) to yield **41b** (456.6 mg, 0.7296 mmol, 50%, colorless oil, (75% (2 steps)).

^{1}H NMR ($CDCl_3$, 400 MHz): 5.416−5.364 (1H, m), 4.380−4.472 (1H, m), 4.277−4.223 (2H, m), 4.141−4.077 (1H, m), 3.903−3.864 (1H, m), 3.608−3.514 (2H, m), 3.386−3.327 (1H, m), 3.270−3.255 (1H, m), 2.224−2.120 (1H, m), 1.886−1.802 (1H, m), 1.581−1.513 (1H, m), 1.386−1.329 (28H, m), 0.773−0.768 (9H, m), −0.047 (3H, s), −0.061 (3H, s). ^{13}C NMR ($CDCl_3$, 100 MHz): 168.29, 168.27, 155.11, 83.36, 83.27, 83.20, 82.37, 79.63, 79.61, 79.52, 79.45, 71.66, 71.59, 71.54, 67.83, 67.77, 67.18, 67.12, 67.04, 63.10, 63.73, 54.36, 54.28, 29.76, 29.73, 29.71, 29.69, 28.71, 28.54, 28.21, 27.85, 27.02, 25.82, 25.75, 20.22, 18.20, 18.18, 14.08, −5.36, −5.40. ^{31}P NMR ($CDCl_3$, 161 MHz): −5.43, −5.98. HRMS (ESI-TOF [M + Na]$^+$): Calcd for $C_{28}H_{56}NNaO_{10}PSi^+$: 648.3303. Found 648.3307. Anal. Calcd for $C_{28}H_{56}NO_{10}PSi$; C, 53.74; H, 9.02; N, 2.24. Found C, 53.61; H, 9.08; N, 2.20.

*tert-Butyl O-(tert-butoxy(((2R,3S)-2-(hydroxymethyl)tetrahydro-2H-pyran-3-yl)oxy)-phosphoryl)-N-(tert-butoxycarbonyl)-L-serinate (**42a**).* Compound **41a** (203.1 mg, 0.3245 mmol) was dissolved in MeOH (8 mL) and cooled to 0 °C Amberlyst15 (1.0155 g) was added to the solution above and the reaction mixture was stirred at 0 °C under Ar atmosphere for 10 min. After 15 min, the reaction mixture was stirred at room temperature under Ar atmosphere for 13 h. After 13 h, the reaction mixture was filtered on Celite and the filtrate was evaporated and the residue was purified by column chromatography (ethyl acetate) to yield **42a** (121.3 mg, 0.2371 mmol, 73%, white sticky solid).

^{1}H NMR ($CDCl_3$, 400 MHz): 5.524−5.505 (1H, m), 4.331−4.127 (4H, m), 3.927−3.888 (1H, m), 3.794−3.687 (2H, m), 3.322 (1H, dt, *J* = 3.6 Hz, 11.2 Hz), 3.172−3.134 (1H, m), 2.876 (1H, brs), 2.277−2.227 (1H, m), 1.734−1.542 (3H, m), 1.456−1.438 (18H, m), 1.408 (9H, s). ^{13}C NMR ($CDCl_3$, 100 MHz): 168.26, 155.19, 84.46, 84.39, 82.78, 82.72, 80.79, 80.72, 79.97, 77.39, 77.07, 76.76, 71.84, 71.79, 67.68, 67.65, 67.56, 67.51, 61.77, 61.64, 54.41, 54.32, 30,78, 30.75, 30.72, 29.79, 29.77, 29.76, 29.73, 28.29, 27.93, 27.92, 25,25. ^{31}P NMR ($CDCl_3$, 161 MHz): −5.18, −5.33. HRMS (ESI-TOF [M + Na]$^+$): Calcd for $C_{22}H_{42}NNaO_{10}P^+$: 534.2439. Found 534.2409. Anal. Calcd for $C_{22}H_{42}NO_{10}P$ + 0.2CH_2Cl_2; C, 50.47; H, 8.03; N, 2.65. Found C, 50.40; H, 8.17; N, 2.51.

*tert-Butyl O-(tert-butoxy(((2R,3R)-2-(hydroxymethyl)tetrahydro-2H-pyran-3-yl)oxy)-phosphoryl)-N-(tert-butoxycarbonyl)-L-serinate (**42b**).* Compound **41b** (428.2 mg, 0.6842 mmol) was dissolved in MeOH (17 mL) and cooled to 0 °C. Amberlyst15 (2.0021 g) was added to the solution above, and stirred at 0 °C under Ar for 10 min. After 10 min, the reaction mixture was stirred at room temperature under Ar for 16 h. After 16 h, reaction mixture was filtered on Celite and the filtrate was evaporated. The residue was purified by column chromatography (ethyl acetate) to yield **42b** (275.2 mg, 0.5380 mmol, 79%, colorless oil).

^{1}H NMR ($CDCl_3$, 400 MHz): 5.528−5.509 (0.4H, m), 5.388−5.368 (0.6H, m), 4.455−4.411 (1H, m), 4.285−4.239 (2H, m), 4.199−4.103 (m, 1H), 3.898−3.863 (1H, m), 3.699 (1H, brs), 3.553−3.470 (2H, m), 3.414−3.349 (2H, m), 2.094−1.981 (1H, m), 1.925−1.771 (1H, m), 1.649−1.540 (1H, m), 1.430−1.382 (18H, m), 1.351−1.307 (10H, m). ^{13}C NMR ($CDCl_3$, 100 MHz): 168.36, 168.21, 155.23, 155.08, 84.64, 84.56, 83.84, 83.76, 82.72, 79.90, 79.82, 78.12, 78.08, 70.79, 70.74, 70.45, 70.39, 67.79, 67.76, 67.46, 67.40, 60.71, 60.50, 54.32, 54.24, 29.75, 29.71, 29.70, 29.65, 28.67, 28.64, 28.55, 28.53, 28.21, 27.84, 20.29, 20.27, 14.10. ^{31}P NMR ($CDCl_3$, 161 MHz): −3.49, −4.01. HRMS (ESI-TOF [M + Na]$^+$): Calcd for $C_{22}H_{42}NNaO_{10}P^+$: 534.2439. Found 534.2414. Anal. Calcd for $C_{22}H_{42}NO_{10}P$; C, 51.65; H, 8.28; N, 2.74. Found C, 51.58; H, 7.98; N, 2.57.

*((2R,3S)-3-((tert-Butoxy((S)-3-(tert-butoxy)-2-((tert-butoxycarbonyl)amino)-3-oxopropoxy)phosphoryl)oxy)tetrahydro-2H-pyran-2-yl)methyl oleate (**43a**).* Compound **42a** (122.6 mg, 0.2397 mmol) was dissolved in CH_2Cl_2 (3 mL) and *N*, *N*-dimethylaminopyridine (87.8 mg, 0.7190 mmol) was added to the solution above. Oleoyl chloride (108.2 mg, 0.3595 mmol) was added to the mixture at rt and stirred at rt under Ar atmosphere for 2.5 h. The reaction mixture was quenched by H_2O (7 mL) and the whole was extracted with CH_2Cl_2 (5 mL × 2). Combined organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography (*n*-hexane: ethyl acetate =2:1) to yield **43a** (120.9 mg, 0.1558 mmol, 65%, translucent oil).

^{1}H NMR ($CDCl_3$, 400 MHz): 5.670−5.650 (0.3H, m), 5.486−5.466 (0.7H, m), 5.356−5.273 (2H, m), 4.423 (1H, dd, *J* = 12.0 Hz, 2.0 Hz), 4.358−4.283 (2H, m), 4.246−4.089 (2H, m), 4.061 (1H, dd, *J* = 12.0 Hz, 6.0 Hz), 3.935−3.902 (1H, m), 3.409−3.304 (2H, m), 2.373−2.312 (3H, m), 2.031−1.957 (4H, m), 1.727−1.561 (5H, m), 1.477−1.427 (27H, m), 1.294−1.242 (20H, m), 0.875−0.837 (3H, m). ^{13}C NMR ($CDCl_3$, 100 MHz): 173.62, 173.60, 168.28, 155.19, 130.18, 130.03, 129.94, 129.74, 128.00, 127.89, 84.03, 83.96, 82.65, 82.59, 79.89, 79.79, 78.44, 78.32, 78.23, 72.67, 72.61, 72.16, 72.10, 67.62, 67.55, 67.44, 67.38, 63.56, 54.39, 54.31, 34.08, 31.90, 30.81, 29.82, 29.79, 29.78, 29.75, 29.70, 29.69, 29.66, 29.63, 29.59, 29.49, 29.33, 29.32, 29.30, 29.27, 29.18, 29.13, 29.12, 29.00, 28.96, 28.32, 27.95, 27.94, 27.19, 27.17, 24.96, 24.94, 24.84, 22.66, 22.55, 14.09, 14.05. ^{31}P NMR ($CDCl_3$, 161 MHz): −6.27, −6.32. HRMS (ESI-TOF [M + Na]$^+$): Calcd for $C_{40}H_{74}NNaO_{11}P^+$: 798.4892. Found 798.4891. Anal. Calcd for $C_{40}H_{74}NO_{11}P$ + 0.2CH_2Cl_2; C, 60.92; H, 9.40; N, 1.77. Found C, 61.14; H, 9.12; N, 1.82.

((2R,3R)-3-((tert-Butoxy((S)-3-(tert-butoxy)-2-((tert-butoxycarbonyl)amino)-3-oxopropoxy)phosphoryl)oxy)tetrahydro-

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*2H-pyran-2-yl)methyl oleate (**43b**).* Compound **42b** (99.4 mg, 0.1943 mmol) was dissolved in CH_2Cl_2 (2.5 mL) and *N,N*-dimethylaminopyridine (71.2 mg, 0.5829 mmol) was added to the solution. The whole was cooled to 0 °C and oleoyl chloride (87.7 mg, 0.2915 mmol) was added under Ar. The reaction mixture was stirred at room temperature under Ar for 3 h. After 3 h, methanol (2 mL) was added and stirred at room temperature for 16 h. After 16 h, solvent was removed under vacuum and the residue was purified by column chromatography (*n*-hexane: ethyl acetate =4:1 to 2:1) to yield **43b** (115.6 mg, 0.1490 mmol, 77%, colorless oil).

^{1}H NMR ($CDCl_3$, 400 MHz): 5.618−5.598 (0.3H, m), 5.492−5.471 (0.5H, m), 5.388−5.266 (2H, m), 4.444−4.405 (1H, m), 4.358−4.313 (2H, m), 4.299−4.172 (1H, m), 4.161−4.068 (2H, m), 4.023−3.984 (m, 1H), 3.612−3.574 (m, 1H), 3.487−3.422 (m, 1H), 2.333−2.172 (m, 3H), 2.027−1.911 (5H, m), 1.707−1.552 (3H, m), 1.480−1.419 (28H, m), 1.293−1.233 (20H, m), 0.872−0.837 (3H, m). ^{13}C NMR ($CDCl_3$, 100 MHz): 173.58, 168.35, 155.22, 129.97, 129.95, 129.72, 83.88, 83.81, 82.64, 82.61, 79.88, 79.80, 76.36, 76.29, 71.86, 71.80, 71.59, 71.54, 67.83, 67.75, 64.37, 64.21, 54.43, 54.35, 34.09, 31.88, 29.85, 29.81, 29.77, 29.74, 29.69, 29.68, 29.49, 29.33, 29.32, 29.30, 29.17, 29.15, 29.11, 29.09, 29.07, 28.96, 28.31, 27.95, 27.93, 27.20, 27.15, 24.87, 24.84, 22.66, 22.55, 20.08, 20.06, 14.09. ^{31}P NMR ($CDCl_3$, 161 MHz): −5.29, −5.82. HRMS (ESI-TOF [M + Na]$^+$): Calcd for $C_{40}H_{74}NNaO_{11}P^+$: 798.4892. Found 798.4891. Anal. Calcd for $C_{40}H_{74}NO_{11}P$+0.1CH_2Cl_2; C, 61.43; H, 9.46; N, 1.79. Found C, 61.46; H, 9.36; N, 1.77.

*tert-Butyl O-(tert-butoxy(((2R,3S)-2-(((3-(2-(undecyloxy)phenyl)-propanoyl)oxy)methyl)-tetrahydro-2H-pyran-3-yl)oxy)phosphoryl)-N-(tert-butoxycarbonyl)-L-serinate (**44a**).* Compound **42a** (94.0 mg, 0.1838 mmol) and carboxylic acid derivative (**B**) (76.6 mg, 0.2389 mmol) were dissolved in CH_2Cl_2 (1 mL). EDCI (45.8 mg, 0.2389 mmol) and DMAP (2.9 mg, 0.0239 mmol) were added to the solution above at rt. Reaction mixture was stirred at room temperature under Ar atmosphere for 48 h. EDCI (1.3 equiv) and MeOH (1 mL) were added to the reaction mixture and stirred at room temperature for 2 h. H_2O (5 mL) was added to the reaction mixture and extracted with CH_2Cl_2 (5 mL × 3). Organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography (*n*-hexane: ethyl acetate =2:1) to yield **44a** (53.2 mg, 0.0654 mmol, 36%, colorless oil).

^{1}H NMR ($CDCl_3$, 400 MHz): 7.158−7.119 (2H, m), 6.844−6.785 (2H, m), 5.705−5.684 (0.3H, m), 5.503−5.483 (0.7H, m), 4.458 (1H, dd, *J* = 2.0 Hz, 12.0 Hz), 4.373−4.305 (2H, m), 4.255−4.170 (1H, m), 4.154−4.047 (2H, m), 3.950−3.904 (3H, m), 3.393−3.307 (2H, m), 2.957−2.918 (2H, m), 2.376−2.345 (2H, m), 1.798−1.568 (5H, m), 1.488−1.259 (43H, m), 0.887−0.853 (3H, m). ^{13}C NMR ($CDCl_3$, 100 MHz): 173.22, 173.20, 168.30, 156.96, 155.32, 155.21, 129.95, 129.01, 128.96, 127.40, 127.38, 120.12, 110.92, 84.05, 83.98, 82.66, 82.61, 79.91, 79.81, 78.47, 78.38, 78.35, 78.26, 77.25, 72.77, 72.70, 72.22, 72.16, 67.75, 67.58, 67.49, 67.40, 63.75, 54.41, 54.32, 33.92, 31.91, 30.95, 30.81, 29.83, 29.80, 29.76, 29.63, 29.61, 29.58, 29.38, 29.33, 29.31, 28.33, 27.97, 27.95, 26.10, 25.91, 25.75, 24.97, 24.94, 22.67, 14.11. ^{31}P NMR ($CDCl_3$, 161 MHz): −6.26, −6.29. HRMS (ESI-TOF [M + Na]$^+$): Calcd for $C_{42}H_{72}NNaO_{12}P^+$: 836.4684. Found 836.4686. Anal. Calcd for $C_{42}H_{72}NO_{12}P$; C, 61.97; H, 8.92; N, 1.72. Found C, 61.70; H, 8.68; N, 1.74.

*tert-Butyl O-(tert-butoxy(((2R,3R)-2-(((3-(2-(undecyloxy)phenyl)-propanoyl)oxy)methyl) tetrahydro-2H-pyran-3-yl)oxy)phosphoryl)-N-(tert-butoxycarbonyl)-L-serinate (**44b**).* Compound **42b** (117.8 mg, 0.2303 mmol) and carboxylic acid derivative (**B**) (95.9 mg, 0.2994 mmol) were dissolved in CH_2Cl_2 (4.3 mL) EDCI (57.4 mg, 0.2994 mmol) and *N,N*-dimethylaminopyridine (7.4 mg, 0.0606 mmol) were added to the reaction mixture. This was stirred at room temperature under Ar for 3 days. MeOH (1.5 mL) and EDCI (48.6 mg, 0.2533 mmol) were added to the reaction mixture and stirred at room temperature under Ar for 1.5 h. H_2O (15 mL) was added to the reaction mixture, the whole was washed with 5% aqueous $KHSO_4$ solution (15 mL) and extracted with CH_2Cl_2 (8 mLX3). Combined organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography (*n*-hexane: ethyl acetate =2:1 to 1:1) to yield **44b** (120.1 mg, 0.1475 mmol, 64%, colorless oil).

^{1}H NMR ($CDCl_3$, 400 MHz): 7.152−7.100 (2H, m), 6.835−6.779 (2H, m), 5.637−5.617 (0.3H, m), 5.508−5.487 (0.5H, m), 4.408−4.315 (3H, m), 4.233−4.103 (3H, m), 4.016−3.976 (1H, m), 3.926 (2H, t, *J* = 6.4 Hz), 3.556−3.520 (1H, m), 3.439 (1H, dt, *J* = 12.0 Hz, 2.0 Hz), 2.942−2.897 (2H, m), 2.663−2.610 (2H, m), 2.264−2.170 (1H, m), 2.047−1.907 (1H, m), 1.802−1.732 (2H, m), 1.688−1.603 (1H, m), 1.477−1.417 (30H, m), 1.378−1.250 (14H, m), 0.862 (3H, t, *J* = 6.4 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): 173.16, 168.35, 156.96, 155.29, 155.23, 129.93, 128.84, 128.79, 127.48, 127.44, 120.10, 110.95, 83.84, 83.77, 82.64, 82.61, 79.87, 79.80, 76.50, 76.42, 76.38, 76.31, 71.92, 71.86, 71.61, 71.56, 67.83, 67.73, 67.41, 64.41, 64.37, 54.52, 54.44, 54.35, 33.94, 33.92, 31.89, 29.85, 29.81, 29.76, 29.61, 29.58, 29.37, 29.33, 29.30, 28.76, 28.66, 28.31, 27.94, 27.93, 26.11, 26.06, 26.02, 22.66, 20.08, 20.06, 14.10.

^{31}P NMR ($CDCl_3$, 161 MHz): −5.29, −5.85. HRMS (ESI-TOF [M + Na]$^+$): Calcd for $C_{42}H_{72}NNaO_{12}P^+$: 836.4684. Found 836.4685. Anal. Calcd for $C_{42}H_{72}NO_{12}P$+0.4H_2O; C, 61.45; H, 8.88; N, 1.71. Found C, 61.09; H, 8.66; N, 1.73.

*tert-Butyl O-(tert-butoxy(((2R,3S)-2-(((3-(2-((3-phenoxybenzyl)-oxy)phenyl)propanoyl)-oxy)methyl)tetrahydro-2H-pyran-3-yl)oxy)-phosphoryl)-N-(tert-butoxycarbonyl)-L-serinate (**45a**).* Compound **42a** (54.4 mg, 0.1063 mmol) and carboxylic acid derivative (**C**) (48.2 mg, 0.1382 mmol) were dissolved in CH_2Cl_2 (2 mL). EDCI (26.5 mg, 0.1382 mmol) and DMAP (1.7 mg, 0.0138 mmol) were added to the solution above at rt. Reaction mixture was stirred at room temperature under Ar atmosphere for 24 h. EDCI (0.3 equiv) and MeOH (1 mL) were added to the reaction mixture and stirred at room temperature for 1 h. H_2O (5 mL) was added to the reaction mixture and extracted with CH_2Cl_2 (3 mLx 3). Organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography (*n*-hexane: ethyl acetate =2:1 to 1:1) to yield crude **45a** (69.1 mg, 0.0821 mmol, 77%, colorless oil).

^{1}H NMR ($CDCl_3$, 400 MHz): 7.356−7.312 (3H, m), 7.198−7.086 (4H, m), 7.046−7.000 (3H, m), 6.954−6.925 (1H, m), 6.930 (1H, dd, *J* = 1.2 Hz, 2.4 Hz), 6.873 (1H, dt, *J* = 0.8 Hz, 7.6 Hz), 6.844−6.824 (1H, m), 5.708−5.688 (0.3H, m), 5.508−5.487 (0.7H, m), 5.050 (2H, s), 4.458 (1H, dd, *J* = 2.0 Hz, 1.2 Hz), 4.379−4.302 (2H, m), 4.265−4.164 (1H, m), 4.152−4.047 (2H, m), 3.928−3.895 (1H, m), 3.390−3.296 (2H, m), 3.006−2.960 (2H, m), 2.689−2.652 (2H, m), 2.373−2.343 (1H, m), 1.726−1.565 (3H, m), 1.489−1.438 (27H, m). ^{13}C NMR ($CDCl_3$, 100 MHz): 173.09, 168.30, 157.64, 156.89, 156.39, 155.22, 139.36, 139.35, 130.17, 129.94, 129.80, 129.26, 129.20, 127.47, 127.45, 123.48, 121.56, 120.80, 119.19, 117.87, 117.05, 111.54, 84.12, 84.04, 82.68, 82.63, 79.92, 78.33, 78.23, 72.24, 72.19, 69.32, 67.56, 67.47, 63.79, 54.41, 54.33, 33.89, 30.94, 29.83, 29.79, 29.75, 28.34, 27.97, 27.96, 25.87, 24.96, 24.93.

^{31}P NMR ($CDCl_3$, 161 MHz): −6.28, −6.33. HRMS (ESI-TOF [M + Na]$^+$): Calcd for $C_{44}H_{60}NNaO_{13}P^+$: 864.3694. Found 864.3689.

*tert-Butyl O-(tert-butoxy(((2R,3R)-2-(((3-(2-((3-phenoxybenzyl)-oxy)phenyl)propanoyl)-oxy)methyl)tetrahydro-2H-pyran-3-yl)oxy)-phosphoryl)-N-(tert-butoxycarbonyl)-L-serinate (**45b**).* Compound **42b** (98.1 mg, 0.1918 mmol) and carboxylic acid derivative (**C**) (86.9 mg, 0.2493 mmol) were dissolved in CH_2Cl_2 (43.6 mL) EDCI (47.8 mg, 0.2493 mmol) and *N,N*-dimethylaminopyridine (3.0 mg, 0.0249 mmol) were added to the reaction mixture. This was stirred at room temperature under Ar for 3 days. MeOH (2 mL) and EDCI (40.4 mg, 0.2110 mmol) were added to the reaction mixture and stirred at room temperature under Ar for 1.5 h. H_2O (15 mL) was added to the reaction mixture, the whole was washed with 5% aqueous $KHSO_4$ solution (15 mL) and extracted with CH_2Cl_2 (8 mLX3). Combined organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography (*n*-hexane: ethyl acetate =2:1 to 1:1) to yield **45b** (79.8 mg, 0.0948 mmol, 49%, colorless oil).

^{1}H NMR ($CDCl_3$, 400 MHz): 7.359−7.308 (3H, m), 7.179−7.085 (4H, m), 7.056−7.003 (3H, m), 6.937 (1H, dd, *J* = 8.0 Hz, 2.0 Hz), 6.892−6.831 (2H, m), 5.644−5.624 (0.3H, m), 5.517−5.496 (0.5H, m), 5.049 (2H, s), 4.373−4.326 (3H, m), 4.247−4.192 (1H, m),

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4.174−4.086 (2H, m), 4.013−3.973 (1H, m), 3.547−3.512 (1H, m), 3.460−3.396 (1H, m), 2.992−2.944 (2H, m), 2.675−2.620 (2H, m), 2.263−2.166 (1H, m), 2.025−1.899 (1H, m), 1.677−1.578 (1H, m), 1.487−1.375 (28H, m). ^{13}C NMR ($CDCl_3$, 100 MHz): 173.04, 168.37, 157.66, 157.64, 156.89, 156.39, 155.25, 139.34, 139.32, 130.18, 130.16, 129.93, 129.92, 129.81, 129.12, 129.06, 127.56, 127.52, 123.49, 121.57, 120.80, 119.18, 117.88, 117.09, 111.57, 83.80, 82.67, 82.64, 79.90, 79.84, 76.47, 76.39, 76.34, 76.27, 71.94, 71.87, 71.63, 71.57, 69.31, 67.80, 67.72, 67.44, 64.49, 64.44, 54.45, 54.36, 33.94, 33.92, 29.87, 29.82, 29.77, 28.76, 28.65, 28.33, 27.95, 26.04, 26.00, 20.00, 14.20. ^{31}P NMR ($CDCl_3$, 161 MHz): −5.27, −5.85. HRMS (ESI-TOF $[M + Na]^+$): Calcd for $C_{44}H_{60}NNaO_{13}P^+$: 864.3694. Found 864.3690. Anal. Calcd for $C_{44}H_{60}NO_{13}P + 2.5H_2O$; C, 59.58; H, 7.39; N, 1.58. Found C, 59.53; H, 7.38; N, 1.71.

*tert-Butyl O-(tert-butoxy(((2R,3R)-2-(((3-(2-((3-(4-chlorophenoxy)benzyl)oxy)phenyl)-propanoyl)oxy)methyl)-tetrahydro-2H-pyran-3-yl)oxy)phosphoryl)-N-(tert-butoxycarbonyl)-L-serinate (**45c**).* Compound (**42b**) (29.4 mg, 0.0594 mmol) and **79b** (21.4 mg, 0.0594 mmol) was dissolved in CH_2Cl_2 (0.7 mL) and DIC (9.2 mg, 0.687 8 mol) and DMAP (2.1 mg, 0.0168 mmol) was added to the solution above. The reaction mixture was stirred at r.t. under Ar for 17 h. After 17 h, solvent was removed under vacuum and purified by column chromatography (*n*-hexane: ethyl acetate =4:1 (120 mL) to 2:1 (300 mL) to 1:1 (200 mL)) to yield **45c** (18.5 mg, 0.0211 mmol, 36%, colorless oil).

^{1}H NMR ($CDCl_3$, 400 MHz): 7.344 (t, 1H, *J* = 8.0 Hz), 7.308−7.266 (m, 2H), 7.182−7.129 (m, 3H), 7.039−7.035 (m, 1H), 6.967−6.829 (m, 5H), 5.642−5.496 (m, 1H), 5.057 (s, 2H), 4.409−4.333 (m, 3H), 4.247−4.100 (m, 3H), 4.018−3.979 (m, 1H), 3.556−3.520 (m, 1H), 3.461−3.402 (m, 1H), 2.995−2.947 (M, 2H), 2.651 (dd, 2H, J = 15.2 and 7.2 Hz), 2.265−2.168 (m, 1H), 2.041−1.900 (m, 1H), 1.687−1.600 (m, 1H), 1.488−1.422 (m, 27H), 1.392−1.387 (m, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): 173.03, 168.38, 157.29, 156.34, 155.62, 155.26, 139.52, 139.50, 130.17, 130.06, 129.79, 129.12, 129.07, 128.48, 127.56, 127.52, 121.99, 120.87, 120.35, 117.94, 117.16, 111.57, 83.92, 82.69, 82.67, 79.93, 76.46, 76.39, 76.36, 76.28, 71.64, 71.59, 69.25, 67.81, 67.72, 67.43, 64.54, 64.46, 54.45, 54.36, 33.92, 29.87, 29.82, 29.77, 28.76. 28.65, 28.32, 27.95, 26.00, 25.97, 20.05. ^{31}P NMR ($CDCl_3$, 161 MHz): −5.28, −5.87.

HRMS (ESI-TOF $[M + Na]^+$): Calcd for $C_{44}H_{59}ClNNaO_{13}P^+$ 898.3305. Found 898.3282. Anal. Calcd for $C_{44}H_{59}ClNO_{13}P$; C, 58.64; H, 6.58; N, 1.54. Found C, 58.84; H, 6.67; N, 1.65.

*tert-Butyl O-(tert-butoxy(((2R,3R)-2-(((3-(2-((3-(p-tolyloxy)-benzyl)oxy)phenyl)-propanoyl)oxy)methyl)tetrahydro-2H-pyran-3-yl)oxy)phosphoryl)-N-(tert-butoxycarbonyl)-L-serinate (**45d**).* Compound **42b** (37.0 mg, 0.0704 mmol) and **79c** (25.5 mg, 0.0704 mmol) was dissolved in CH_2Cl_2 (1 mL) and DIC (11.5 mg, 0.0915 mol) and DMAP (2.6 mg, 0.0211 mmol) was added to the solution above. The reaction mixture was stirred at r.t. under Ar for 15 h. After 15 h, solvent was removed under vacuum and purified by column chromatography (*n*-hexane: ethyl acetate =4:1 (100 mL) to 2:1 (300 mL) to 1:1 (200 mL)) to yield **45d** (31.9 mg, 0.0373 mmol, 53%, colorless oil).

^{1}H NMR ($CDCl_3$, 400 MHz): 7.326−7.287 (m, 1H), 7.177−7.110 (m, 5H), 7.016 (m, 1H), 6.940−6.830 (m, 5H), 5.646−5.497 (m, 1H), 5.040 (s, 2H), 4.401−4.277 (m, 3H), 4.241−4.196 (m, 1H), 4.177−4.091 (m, 2H), 4.015−3.976 (m, 1H), 3.552−3.516 (m, 1H), 3.462−3.398 (m, 1H), 2.993−2.946 (m, 2H), 2.678−2.623, 2.335 (s, 3H), 2.263−2.166 (m, 1H), 2.026−1.898 (m, 1H), 1.679−1.579 (m, 1H), 1.488−1.423 (m, 27H), 1.393−1.376 (m, 1H).

^{13}C NMR ($CDCl_3$, 100 MHz): 173.07, 158.22, 156.42, 154.35, 139.21, 133.19, 130.30, 130.15, 129.83, 129.06, 127.55, 127.52, 121.16, 120.77, 119.41, 117.33, 116.58, 111.56, 82.68, 79.91, 76.36, 76.28, 71.89, 71.64, 69.35, 67.80, 67.72, 67.42, 64.50, 54.45, 33.94, 29.86, 29.82, 29.77, 28.76, 28.32, 27.95, 26.05, 26.01, 20.72, 20.06. ^{31}P NMR ($CDCl_3$, 161 MHz): −5.28, −5.87. HRMS (ESI-TOF $[M + Na]^+$): Calcd for $C_{45}H_{62}NNaO_{13}P^+$ 878.3851. Found 878.3831. Anal. Calcd for $C_{45}H_{62}NO_{13}P + CH_2Cl_2$; C, 58.72; H, 6.86; N, 1.49. Found C, 58.76; H, 6.86; N, 1.65.

*(2R,3S)-2-(Hydroxymethyl)-3,6-dihydro-2H-pyran-3-ol (**46a**).* Compound **36a** (784.5 mg, 3.6621 mmol) was dissolved in MeOH (10 mL) and sodium methoxide (98.9 mg, 1.8311 mmol) was added to the solution above. The reaction mixture was stirred at room temperature under Ar atmosphere for 10 min. After 10 min, solvent was evaporated and the residue was dissolved in chloroform. This solution was filtered on Celite and the filtrate was evaporated to yield **46a** (414.6 mg, 3.1858 mmol, 87%, yellow oil).

^{1}H NMR ($CDCl_3$, 400 MHz): 5.848−5.781 (2H, m), 4.190−4.101 (3H, m), 3.874 (1H, dd, *J* = 11.6 Hz, 3.6 Hz), 3.785 (1H, dd, *J* = 11.6 Hz, 5.6 Hz), 3.354−3.306 (1H, m), 2.622 (1H, s). ^{13}C NMR ($CDCl_3$, 100 MHz): 128.54, 127.62, 78.52, 65.35, 64.15, 63.03. HRMS (ESI-TOF $[M + Na]^+$): Calcd for $C_6H_{10}NaO_3^+$: 153.0522. Found 153.0549.

*(2R,3R)-2-(Hydroxymethyl)-3,6-dihydro-2H-pyran-3-ol (**46b**).* Compound **36b** (543.0 mg, 2.5348 mmol) was dissolved in methanol (5 mL), sodium methoxide (68.5 mg, 1.2674 mmol) was added to the solution above. The reaction mixture was stirred at room temperature under Ar for 1.5 h. After 1.5 h, the reaction mixture was evaporated to remove solvent and the residue was dissolved in chloroform. The solution was filtered on Celite and filtrate was evaporated to yield crude **46b** (329.3 mg, 2.5301 mmol, 100%, colorless sticky oil).

^{1}H NMR ($CDCl_3$, 400 MHz): 6.057−6.009 (1H, m), 5.987−5.949 (1H, m), 4,281 (1H, dddd, *J* = 16.8 Hz, 11.6 Hz 3.6 Hz, 1.6 Hz), 4.198−4.142 (1H, m), 3.949−3.882 (2H, m), 3.811 (1H, dd, *J* = 11.6 Hz, 4.4 Hz), 3.577 (1H, spt, *J* = 2.0 Hz, 2.4 Hz, 6.4 Hz), 2.454−2.233 (2H, m). ^{13}C NMR ($CDCl_3$, 100 MHz): 130.48, 126.42, 77.97, 66.15, 63.38. 62.98. HRMS (ESI-TOF $[M + Na]^+$): Calcd for $C_6H_{10}NaO_3^+$: 153.0522. Found 153.0541.

*(2R,3S)-2-(((tert-Butyldimethylsilyl)oxy)methyl)-3,6-dihydro-2H-pyran-3-ol (**47a**).* Compound **46a** (445.6 mg, 3.4340 mmol) was dissolved in CH_2Cl_2 (10 mL) and imidiazole was added to the solution. This was cooled to 0 °C and *tert*-butyldimethylsilyl chloride (567.7 mg, 3.7667 mmol) was added. This reaction mixture was stirred at 0 °C under Ar atmosphere for 10 min and stirred at room temperature for 15 h. After 15 h, water (20 mL) was added to the reaction mixture and the whole was extracted with CH_2Cl_2 (15 mLX3). Combined organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography to yield **47a** (594.1 mg, 2.4309 mmol, 71%, colorless oil).

^{1}H NMR ($CDCl_3$, 400 MHz): 5.839−5.773 (2H, m), 4.209−4.186 (1H, m), 4.158−4.082 (2H, m), 3.964−3.921 (1H, m), 3.750−3.705 (1H, m), 3.395−3.338 (1H, m), 3.049 (1H, brs), 0.908 (9H, s), 0.108 (6H, s). ^{13}C NMR ($CDCl_3$, 100 MHz): 127.92, 127.08, 76.70, 67.33, 65.92, 65.29, 25.86, 18.24, −5.51, −5.57. HRMS (ESI-TOF $[M + Na]^+$): Calcd for $C_{12}H_{24}NaO_3Si^+$: 267.1387, Found 267.1370. Anal. Calcd for $C_{12}H_{24}O_3Si$; C, 58.97; H, 9.90; N, 0.00. Found C, 58.89; H, 9.79; N, 0.00.

*(2R,3R)-2-(((tert-Butyldimethylsilyl)oxy)methyl)-3,6-dihydro-2H-pyran-3-ol (**47b**).* Compound **46b** (460.6 mg, 3.5347 mmol) was dissolved in CH_2Cl_2 (10 mL) and imidazole (529.4 mg, 7.7762 mmol) was added to the solution above. This solution was cooled to 0 °C and *tert*-butyldimethylsilyl chloride (639.3 mg, 4.2416 mmol) was added. The reaction mixture was stirred at 0 °C under Ar for 10 min and stirred at room temperature under Ar for 21 h. The reaction mixture was quenched by water (20 mL) and the whole was extracted with CH_2Cl_2 (15 mL X3). Combined organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by column chromatography (*n*-hexane: ethyl acetate =1:1 to ethyl acetate) to yield **47b** (275.2 mg, 1.1260 mmol, 79%, colorless oil).

^{1}H NMR ($CDCl_3$, 400 MHz): 6.073−6.024 (1H, m), 5.941 (1H, dddd, *J* = 10.0 Hz, 3.6 Hz, 2.0 Hz, 1.6 Hz), 4.242 (1H, ddd, *J* = 16.8 Hz, 3.6 Hz, 1.6 Hz), 4.138 (1H, ddd, *J* = 16.8 Hz, 4.0 Hz, 2.0 Hz), 3.982−3.947 (1H, m), 3.859 (1H, dd, *J* = 10.4 Hz, 6.4 Hz), 3.807 (1H, dd, *J* = 10.8 Hz, 6.0 Hz), 3.534 (1H, dt, *J* = 6.4 Hz, 2.0 Hz), 1.978−1.965 (1H, m), 0.900 (9H, s), 0.089−0.084 (6H, m). ^{13}C NMR ($CDCl_3$, 100 MHz): 130.31, 126.62, 78.11, 66.18, 62.87, 62.60, 25.90, 18.31, −5.33, −5.38. HRMS (ESI-TOF $[M + Na]^+$): Calcd for $C_{12}H_{24}NaO_3Si^+$: 267.1387, Found 267.1383. Anal. Calcd for

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$C_{12}H_{24}O_3Si$; C, 58.97; H, 9.90; N, 0.00. Found C, 58.88; H, 9.60; N, 0.00.

*tert-Butyl O-(tert-butoxy(((2R,3S)-2-(((tert-butyldimethylsilyl)-oxy)methyl)-3,6-dihydro-2H-pyran-3-yl)oxy)phosphoryl)-N-(tert-butoxycarbonyl)-L-serinate (**48a**).* **40** (724.1 mg 1.6034 mmol) and compound **47a** (587.8 mg, 2.4051 mmol) was dissolved in dry CH_2Cl_2 (10 mL) and dry toluene (2 mL) and coevaporated to remove containing water. The residue was dissolved in CH_2Cl_2 (10 mL) and *1H*-tetrazole (505.4 mg, 7.2152 mmol) in THF (10 mL) was added to the solution above. This reaction mixture was stirred at room temperature under Ar atmosphere for 21 h. After 21 h, *tert*-butylhydroperoxide 5 M in decane (0.641 mg, 3.2068 mmol) was added to the reaction mixture and stirred at room temperature under Ar atmosphere for 7 h. After 7 h, water (20 mL) was added and the whole was extracted with CH_2Cl_2 (15 mLX2). Organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography (*n*-hexane: ethyl acetate =4:1 to 2:1) to yield **48a** (713.3 mg, 1.1435 mmol, colorless oil, 71% 2 steps). ^{1}H NMR ($CDCl_3$, 400 MHz): 5.980−5.881 (2H, m), 5.495−5.445 (1H, m), 4.711−4.647 (1H, m), 4.364−4.154 (4H, m), 3.927 (1H, dddd, *J* = 11.6 Hz, 4.8 Hz, 4.4 Hz, 2.4 Hz), 3.734 (1H, dd, *J* = 11.6 Hz, 6.4 Hz), 3.516−3.475 (1H, m), 1.646 (1H, brs), 1.548−1.442 (27H, m), 0.899 (9H, s), 0.083−0.077 (6H, m). ^{13}C NMR ($CDCl_3$, 100 MHz): 168.37, 168.32, 155.24, 129.86, 125.15, 125.01. 83.88, 83.81, 82.68, 79/92, 77.88, 77.78, 69.26, 69.20, 68.93, 68.87, 67.97, 67.66, 67.47, 64.95, 64.93, 63.08, 54.53, 54.45, 54.36, 30.91, 29.86, 29.84, 29.81, 29.80, 29.14, 28.33, 27.97, 25.98, 25.93, 23.91, 18.46, 18.45, 14.19. ^{31}P NMR ($CDCl_3$, 161 MHz): −5.79, −5.86. HRMS (ESI-TOF $[M + Na]^+$): Calcd for $C_{28}H_{54}NNaO_{10}PSi^+$: 646.3147, Found 646.3151. Anal. Calcd for $C_{28}H_{54}NO_{10}PSi + 0.1CH_2Cl_2$; C, 53.38; H, 8.64; N, 2.22; Found C, 53.11; H, 8.56; N, 2.24.

*tert-Butyl O-(tert-butoxy(((2R,3R)-2-(((tert-butyldimethylsilyl)-oxy)methyl)-3,6-dihydro-2H-pyran-3-yl)oxy)phosphoryl)-N-(tert-butoxycarbonyl)-L-serinate (**48b**).* **40** (319.8 mg, 0.7081 mmol) and compound **47b** (259.6 mg, 1.0622 mmol) were dissolved in CH_2Cl_2 (2 mL) and toluene (0.5 mL) and evaporated to remove containing water. The residue was dissolved in dry CH_2Cl_2 (2 mL) and *1H*-tetrazole (198.4 mg, 2.8327 mmol) in THF (2 mL) was added to the solution. This reaction mixture was stirred at room temperature under Ar for 2 h. After 2 h, *tert*-butylhydroperoxide (0.2832 mg, 1.4162 mmol) was added to the reaction mixture and stirred at room temperature under Ar for 1.5 h. After 1.5 h, the reaction mixture was quenched by water (20 mL) and the whole was extracted with CH_2Cl_2 (10 mL × 2). Combined organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography (*n*-hexane: ethyl acetate =2:1) to yield **48b** (349.6 mg, 0.5604 mmol, 79% (2 steps), colorless oil). ^{1}H NMR ($CDCl_3$, 400 MHz): 6.125−6.053 (2H, m), 5.513−5.442 (1H, m), 4.629−4.590 (1H, m), 4.381−4.277 (3H, m), 4.218−4.166 (2H, m), 3.858−3.741 (2H, m), 3.597−3.558 (1H, m), 1.490−1.468 (18H, m), 1.441 (9H, s), 0.901−0.883 (9H, m), 0.081−0.060 (6H, m). ^{13}C NMR ($CDCl_3$, 100 MHz): 168.45, 155.26, 132.82, 123.10, 82.58, 82.56, 79.82, 77.66, 77.58, 68.09, 65.80, 65.72, 63.01, 62.67, 54.40, 29.88, 29.87, 29.82, 28.34, 27.98, 27.94, 25.92, 18.36, 18.33, −5.19, −5.20, −5.25, −5.28. ^{31}P NMR ($CDCl_3$, 161 MHz): −5.56, −5.82. HRMS (ESI-TOF $[M + Na]^+$): Calcd for $C_{28}H_{54}NNaO_{10}PSi^+$: 646.3147. Found 646.3160. Anal. Calcd for $C_{28}H_{54}NO_{10}PSi$; C, 53.91; H, 8.73; N, 2.25. Found C, 53.64; H, 8.44; N, 2.35.

*tert-Butyl O-(tert-butoxy(((2R,3S)-2-(hydroxymethyl)-3,6-dihydro-2H-pyran-3-yl)oxy)-phosphoryl)-N-(tert-butoxycarbonyl)-L-serinate (**49a**).* Compound **48a** (660.5 mg, 1.0588 mmol) was dissolved in MeOH (16 mL). Amberlyst15 (3.3025 g) was added to the solution above and the reaction mixture was stirred at room temperature under Ar atmosphere for 11 h. After 11 h, the reaction mixture was filtered on Celite and the filtrate was evaporated and the residue was purified by column chromatography (ethyl acetate) to yield **49a** (322.2 mg, 0.6324 mmol, 61%, white sticky solid). ^{1}H NMR ($CDCl_3$, 400 MHz): 5.873−5.803 (2H, m), 5.581−5.562 (1H, m), 4.823−4.776 (1H, m), 4.313−4.254 (m, 2H), 4.209−4.135 (m, 3H), 3.810−3.705 (m, 2H), 3.407−3.359 (m, 1H), 3.271 (1H, brs), 1.441−1.435 (9H, m), 1.413 (9H, s), 1.380 (9H, s). ^{13}C NMR ($CDCl_3$, 100 MHz): 168.27, 168.20, 155.16, 129.28, 129.23, 125.40, 125.37, 84.39, 84.32, 84.28, 84.20, 82.69, 82.64, 68.66, 68.61, 68.55, 67.50, 65.32, 65.30, 61.56, 61.47, 54.37, 54.2829.72, 29.70, 29.68, 29.65, 28.20, 27.85, 27.84. ^{31}P NMR ($CDCl_3$, 161 MHz): −5.13, −5.31. HRMS (ESI-TOF $[M + Na]^+$): Calcd for $C_{22}H_{40}NNaO_{10}P^+$: 532.2282. Found 532.2283. Anal. Calcd for $C_{22}H_{40}NO_{10}P + 0.2CH_2Cl_2$; C, 50.64; H, 7.73; N, 2.66. Found C, 50.78; H, 7.74; N, 2.67.

*tert-Butyl O-(tert-butoxy(((2R,3R)-2-(hydroxymethyl)-3,6-dihydro-2H-pyran-3-yl)oxy)-phosphoryl)-N-(tert-butoxycarbonyl)-L-serinate (**49b**).* Compound **48b** (285.4 mg, 0.4575 mmol) was dissolved in methanol (7 mL) and this solution was cooled to 0 °C Amberlyst15 (1.4110 g) was added to the solution above, stirred at 0 °C under Ar for 5 min and stirred at room temperature under Ar for 9 h. After 9 h, reaction mixture was filtered on Celite and the filtrate was evaporated. The residue was purified by column chromatography (ethyl acetate) to yield crude **49b** (191.6 mg, 0.3769 mmol, 82%, colorless oil). ^{1}H NMR ($CDCl_3$, 400 MHz): 6.112−6.074 (1H, m), 6.025−5.940 (1H, m), 5.506−5.408 (1H, m), 4.701−4.637 (1H, m), 4.396−4.308 (2H, m), 4.303−4.082 (4H, m), 3.785−3.636 (3H, m), 1.515−1.486 (18H, m), 1.444−1.436 (9H, m). ^{13}C NMR ($CDCl_3$, 100 MHz): 168.31, 168.24, 155.23, 155.14, 133.03, 132.77, 122.50, 122.47, 122.32, 122.29, 84.93, 84.85, 84.23, 84.15, 82.84, 79.99, 79.92, 76.55, 76.53, 76.50, 76.47, 67.87, 67.81, 67.60, 67.55, 67.45, 67.39, 65.70, 65.61, 60.66, 60.30, 54.33, 54.24, 29.77, 29.75, 29.73, 29.71, 28.26, 27.89, 27.87.

^{31}P NMR ($CDCl_3$, 161 MHz): - 3.39, −3.69. HRMS (ESI-TOF $[M + Na]^+$): Calcd for $C_{22}H_{40}NNaO_{10}P^+$: 532.2282. Found: 532.2298.

*((2R,3S)-3-((tert-Butoxy((S)-3-(tert-butoxy)-2-((tert-butoxycarbonyl)amino)-3-oxopropoxy)phosphoryl)oxy)-3,6-dihydro-2H-pyran-2-yl)methyl oleate (**50a**).* Compound **49a** (92.9 mg, 0.1823 mmol) was dissolved in CH_2Cl_2 (10 mL) and *N,N*-dimethylaminopyridine (66.8 mg, 0.5470 mmol) was added to the solution. The whole was cooled to 0 °C and oleoyl chloride (82.3 mg, 0.2735 mmol) was added under Ar. The reaction mixture was stirred at room temperature under Ar for 1.5 h. After 1.5 h, solvent was removed under vacuum and the residue was purified by column chromatography (*n*-hexane: ethyl acetate =4:1 to 2:1) to yield **50a** (101.1 mg, 0.1306 mmol, 72%, colorless oil). ^{1}H NMR ($CDCl_3$, 400 MHz): 5.896−5.840 (2H, m), 5.569−5.549 (0.3H, m), 5.442−5.422 (0.6H, m), 5.311−5.227 (2H, m), 4.711−4.619 (1H, m), 4.411 (1H, dd, *J* = 12.0 Hz, 2.0 Hz), 4.299−255 (2H, m), 4.225−4.506 (4H, m), 3.612 (1H, m), 2.293 (2H, t, *J* = 7.6 Hz), 1.985−1.838 (4H, m), 1.582−1.547 (2H, m), 1.440−1.409 (18H, m), 1.378 (9H, s), 1.229−1.197 (20H, m), 0.826−0.792 (3H, m). ^{13}C NMR ($CDCl_3$, 100 MHz): 173.54, 173.52, 168.31, 168.26, 155.26, 155.20, 130,18, 130.02, 129.95, 129.73, 129.52, 129.49, 128.01, 127.89, 125.14, 124.97, 84.24, 84.17, 82.69, 79.90, 79.84, 77.24, 74.96, 74.88, 74.79, 69.12, 69.06, 68.72, 68.67, 67.58, 67.53, 65.20, 65.16, 63.04, 54.42, 54.33, 34.07, 31.89, 31.87, 31.49, 29.81, 29.76, 29.74, 29.68, 29.66, 29.65, 29.62, 29.58, 29.49, 29.33, 29.31, 29.29, 29.28, 29.26, 29.17.29.16, 29.11, 29.10, 29.07, 28.95, 28.31, 27.95, 27.94, 27.19, 27.1625.60, 24.84, 22.64, 22.62, 22.54, 14.07, 14.04. ^{31}P NMR ($CDCl_3$, 161 MHz): - 5.70, −5.78. HRMS (ESI-TOF $[M + Na]^+$): Calcd for $C_{40}H_{72}NNaO_{11}P^+$ 796.4735. Found 796.4730. Anal. Calcd for $C_{40}H_{72}NO_{11}P + 0.2CH_2Cl_2$; C, 61.04; H, 9.23; N, 1.77. Found C, 61.02; H, 8.95; N, 1.82.

*((2R,3R)-3-((tert-Butoxy((S)-3-(tert-butoxy)-2-((tert-butoxycarbonyl)amino)-3-oxopropoxy)phosphoryl)oxy)-3,6-dihydro-2H-pyran-2-yl)methyl oleate (**50b**).* Compound **49b** (100.6 mg, 0.1974 mmol) was dissolved in CH_2Cl_2 (3 mL) and *N,N*-dimethylaminopyridine (72.4 mg, 0.5923 mmol) was added to the solution. The whole was cooled to 0 °C and oleoyl chloride (89.1 mg, 0.2962 mmol) was added under Ar. The reaction mixture was stirred at 0 °C under Ar for 10 min and stirred at room temperature under Ar for 1 h. After 1 h, solvent was removed under vacuum and the residue was purified by column chromatography (*n*-hexane: ethyl acetate =2:1) to yield **50b** (125.4 mg, 0.1620 mmol, 82%, colorless oil).

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^{1}H NMR ($CDCl_3$, 400 MHz): 6.084−6.015 (2H, m), 5.600−5.447 (1H, m), 5.360−5.262 (2H, m), 4.622−4.590 (1H, m), 4.351−4.057 (6H, m), 3.783−3.759 (1H, m), 2.337−2.288 (2H, m), 2.006−1.948 (4H, m), 1.627−1.554 (2H, m), 1.472−1.410 (27H, m), 1.309−1.204 (21H, m), 0.861−0.827 (3H, m). ^{13}C NMR ($CDCl_3$, 100 MHz): 173.58, 173.55, 173.53, 168.34, 168.32, 155.26, 155.21, 132.59, 132.33, 130.16, 129.99, 129.95, 129.71, 129.70, 128.02, 128.00, 127.87, 84.10, 84.06, 84.03, 83.98, 82.61, 82.59, 79.82, 79.79, 74.42, 74.35, 74.27, 68.17, 68.10, 67.87, 67.82, 67.56, 67.50, 67.43, 65.57, 65.47, 63.71, 63.48, 54.49, 54.38, 54.29, 34.08, 34.07, 31.89. 31.87, 31.49, 29.84, 29.82, 29.79, 29.78, 29.73, 29.70, 29.68, 29.66, 29.48, 29.26, 29.16, 29.14, 29.09, 29.07, 29.0628.30, 27.93, 27.91, 27.18, 27.17, 27.14, 25.60, 24.86, 24.83, 22.64, 22.53, 14.07, 14.04. ^{31}P NMR ($CDCl_3$, 161 MHz): δ - 5.53, −5.67.

HRMS (ESI-TOF [M + Na]$^+$): Calcd for $C_{40}H_{72}NNaO_{11}P^+$: 796.4735. Found 796.4733.

Anal. Calcd for $C_{40}H_{72}NO_{11}P$; C, 62.07; H, 9.38; N, 1.80. Found: C, 61.88; H, 9.35; N, 1.79.

*tert-Butyl O-(tert-butoxy(((2R,3S)-2-(((3-(2-(undecyloxy)phenyl)propanoyl)oxy)methyl)-3,6-dihydro-2H-pyran-3-yl)oxy)phosphoryl)-N-(tert-butoxycarbonyl)-L-serinate (**51a**).* Carboxylic acid derivatives (**B**) (39.1 mg, 0.1219 mmol), compound **49a** (62.1 mg, 0.1219 mmol) were dissolved in CH_2Cl_2 (1 mL) and diisopropylcarbodiimide (20.0 mg, 0.1585 mmol) and *N,N*-dimethylaminopyridine (1.5 mg, 0.0122 mmol) were added to the solution above. The mixture was stirred at room temperature under Ar atmosphere for 16 h. After 16 h, the reaction mixture was quenched by water (7 mL) and extracted with CH_2Cl_2 (7 mL × 2). Combined organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography (*n*-hexane: acetone =3:1) to yield crude **51a** (39.2 mg, 0.0483 mmol, 40%, colorless oil).

^{1}H NMR ($CDCl_3$, 400 MHz): 7.178−7.127 (2H, m), 6.862−6.793 (2H, m), 5.928−5.900 (2H, m), 5.658−5.489 (1H, m), 4.752−4.652 (1H, m), 4.498−4.463 (1H, m), 4.396−4.114 (6H, m), 3.964−3.924 (2H, m), 3.669−3.628 (1H, m), 2.969−2.930 (2H, m), 2.698−2.621 (2H, m), 1.820−1.750 (2H, m), 1.500−1.262 (43H, m), 0.899−0.857 (3H, m). ^{13}C NMR ($CDCl_3$, 100 MHz): 173.16, 168.29, 156.97, 155.24, 129.96, 129.58, 129.54, 128.94, 128.88, 128.84, 127.53, 127.45, 127.43, 125.13, 124.95, 120.13, 110.94, 84.21, 82.72, 79.93, 29.87, 74.91, 74.82, 69.19, 69.14, 68.76, 68.70, 67.75, 67.62, 65.16, 65.11, 63.22, 54.42, 33.92, 31.91, 29.81, 29.79, 29.77, 29.64, 29.62, 29.59, 29.38, 29.34, 29.31, 28.33, 27.97, 27.96, 26.11, 26.05, 25.97, 25.95, 22.68, 14.12. ^{31}P NMR ($CDCl_3$, 161 MHz): −5.48, −5.68. HRMS (ESI-TOF [M + Na]$^+$): Calcd for $C_{42}H_{70}NNaO_{12}P^+$: 834.4533. Found 834.4522.

*tert-Butyl O-(tert-butoxy(((2R,3R)-2-(((3-(2-(undecyloxy)phenyl)propanoyl)oxy)methyl)-3,6-dihydro-2H-pyran-3-yl)oxy)phosphoryl)-N-(tert-butoxycarbonyl)-L-serinate (**51b**).* Carboxylic acid derivatives (**B**) (67.7 mg, 0.2111 mmol), compound **49b** (107.5 mg, 0.2111 mmol) were dissolved in CH_2Cl_2 (2 mL) and diisopropylcarbodiimide (58.6 mg, 0.4644 mmol) and *N,N*-dimethylaminopyridine (8.0 mg, 0.0633 mmol) were added to the solution above. The mixture was stirred at room temperature under Ar atmosphere for 18 h. After 18 h, the reaction mixture was quenched by water (10 mL) and extracted with CH_2Cl_2 (10 mL × 2). Combined organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography (1st: CH_2Cl_2: MeOH = 12:1, second: *n*-hexane: acetone =5:1, third: *n*-hexane: ethyl acetate =2:1) to yield crude **51b** (101.0 mg, 0.1244 mmol, 59%, colorless oil).

^{1}H NMR ($CDCl_3$, 400 MHz): 7.174−7.127 (2H, m), 6.854−6.800 (2H, m), 6.104−6.039 (2H, m), 5.630−5.475 (1H, m), 4.590−4.579 (1H, m), 4.381−4.106 (7H, m), 3.947 (2H, t, J = 6.4 Hz), 3.765−3.732 (1H, m), 2.967−2.923 (2H, m), 2.690−2.642 (2H, m), 1.823−1.753 (2H, m), 1.494−1.264 (43H, m), 0.897−0.860 (3H, m). ^{13}C NMR ($CDCl_3$, 100 MHz): 173.20, 173.19, 168.38, 156.98, 155.26, 132.61, 132.35, 129.95, 128.85, 128.80, 127.52, 127.48, 122.77, 122.73, 120.13, 110.98, 84.08, 82.65, 79.88, 74.51, 74.44, 74.40, 74.32, 68.25, 68.19, 67.86, 67.77, 67.52, 65.60, 65.49, 63.84, 63.69, 54.42, 33.96, 31.91, 29.87, 29.84, 29.83, 29.80, 29.64, 29.63, 29.60, 29.39, 29.35, 29.32, 28.33, 27.97, 27.94, 26.13, 26.08, 26.05, 25.93, 22.68, 14.12. ^{31}P NMR ($CDCl_3$, 161 MHz): −5.54, −5.72. HRMS (ESI-TOF [M + Na]$^+$): Calcd for $C_{42}H_{70}NNaO_{12}P^+$: 834.4533. Found 834.4530.

*tert-Butyl O-(tert-butoxy(((2R,3S)-2-(((3-(2-((3-phenoxybenzyl)oxy)phenyl)propanoyl)-oxy)methyl)-3,6-dihydro-2H-pyran-3-yl)-oxy)phosphoryl)-N-(tert-butoxycarbonyl)-L-serinate (**52a**).* Carboxylic acid derivative (**C**) (68.2 mg, 0.1958 mmol), compound **49a** (99.7 mg, 0.1958 mmol) were dissolved in CH_2Cl_2 (1 mL) and diisopropylcarbodiimide (32.1 mg, 0.2545 mmol) and *N,N*-dimethylaminopyridine (2.4 mg, 0.0196 mmol) were added to the solution above. The mixture was stirred at room temperature under Ar atmosphere for 18 h. After 18 h, the reaction mixture was quenched by water (8 mL) and extracted with CH_2Cl_2 (8 mLX2). Combined organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography (*n*-hexane: acetone =3:1) to yield **52a** (98.9 mg, 0.1178 mmol, 60%, colorless oil).

^{1}H NMR ($CDCl_3$, 400 MHz): 7.358−7.313 (3H, m), 7.191−7.084 (4H, m), 7.041−6.997 (3H, m), 6.951−6.926 (1H, m), 6.891−6.826 (2H, m), 5.920−5.910 (2H, m), 5.669−5.500 (1H, m), 5.048 (2H, s), 4.749−4.648 (1H, m), 4.492−4.456 (1H, m), 4.394−4.322 (2H, m), 4.289−4.185 (1H, m), 4.159−4.107 (3H, m), 3.662−3.619 (1H, m), 3.008−2.965 (2H, m), 2.696−2.658 (2H, m), 1.493−1.436 (27H, m). ^{13}C NMR ($CDCl_3$, 100 MHz): 173.01, 168.34, 168.29, 157.65, 156.88, 156.38, 155.31, 155.23, 139.33, 130.19, 129.94, 129.81, 129.62, 129.58, 129.17, 129.12, 127.52, 127.50, 125.08, 124.93, 123.49, 121.56, 120.82, 119.19, 117.89, 117,04, 111.55, 84.42, 84.34, 84.29, 84.22, 82.72, 79.93, 29.87, 74.98, 74.88, 74.79, 69.31, 69.18, 69.12, 68.76, 68.70, 67.62, 67.56, 65.14, 65.10, 63.30, 63.26, 54.53, 54.44, 54.35,33.89, 29.83, 29.81, 29.76, 28.33, 27.97, 27.96, 25.92. ^{31}P NMR ($CDCl_3$, 161 MHz): 5.64, −5.73.

HRMS (ESI-TOF [M + Na]$^+$): Calcd for $C_{44}H_{58}NNaO_{13}P^+$: 862.3538. Found 862.3521. Anal. Calcd for $C_{44}H_{58}NO_{13}P$; C, 60.33; H, 7.13; N, 1.60. Found C, 60.12; H, 6.91; N, 1.51.

*tert-Butyl O-(tert-butoxy(((2R,3R)-2-(((3-(2-((3-phenoxybenzyl)oxy)phenyl)propanoyl)-oxy)methyl)-3,6-dihydro-2H-pyran-3-yl)-oxy)phosphoryl)-N-(tert-butoxycarbonyl)-L-serinate (**52b**).* Carboxylic acid derivative (**C**) (59.0 mg, 0.1693 mmol), compound **49b** (86.2 mg, 0.1693 mmol) were dissolved in CH_2Cl_2 (2 mL) and diisopropylcarbodiimide (47.0 mg, 0.3724 mmol) and *N,N*-dimethylaminopyridine (2.1 mg, 0.0169 mmol) were added to the above solution. The mixture was stirred at room temperature under Ar atmosphere for 20 h. After 20 h, the reaction mixture was quenched by water (7 mL) and extracted with CH_2Cl_2 (7 mLδ2). Combined organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography (*n*-hexane: acetone =3:1) to yield crude **52b** (43.4 mg, 0.0517 mmol, 31%, colorless oil).

^{1}H NMR ($CDCl_3$, 400 MHz): 7.362−7.313 (3H, m), 7.188−7.090 (4H, m), 7.058 (1H, m), 7.029−7.007 (2H, m), 6.952−6.927 (1H, m), 6.896−6.839 (2H, m), 6.090−6.024 (2H, m), 5.629−5.609 (1H, m), 5.058 (2H, s), 4.582−4.556 (1H, m), 4.375−4.075 (7H, m), 3.740−3.708 (1H, m), 3.004−2.958 (2H, m), 2.689−2.637 (2H, m), 1.482−1.230 (27H, m). ^{13}C NMR ($CDCl_3$, 100 MHz): 173.05, 168.38, 157.66, 156.89, 156.40, 155.25, 139.34, 139.31, 132.62, 132.36, 130.19, 130.16, 129.92, 129.81, 129.10, 129.04, 127.59, 127.55, 123.49, 122.74, 122.69, 121.57, 120.82, 119.18, 117.89, 117.10, 111.57, 84.08, 82.65, 79.87, 74.47, 74.39, 74.35, 74.27, 69.32, 68.25, 67.91, 67.86, 67.61, 65.57, 65.46, 63.87, 63.72, 54.40, 54.32,33.95, 29.87, 29.83, 29.80, 29.34, 27.96, 27.94, 26.07, 26.04. ^{31}P NMR ($CDCl_3$, 161 MHz): −5.52, −5.72. HRMS (ESI-TOF [M + Na]$^+$): Calcd for $C_{44}H_{58}NNaO_{13}P^+$: 862.3538. Found 862.3520.

*Benzene LysoPS analogues. 3-(((tert-Butyldimethylsilyl)oxy)-methyl)phenol (**54b**).* *m*-Hydroxybenzylalcohol **53b** was dissolved in CH_2Cl_2 (2 mL) and THF (1 mL) and imidazole (60.3 mg, 0.8861 mmol) was added to the solution at 0 °C. TBSCl (133.6 mg, 0.8861 mmol) in THF (3 mL) was added dropwise for 30 min. The reaction mixture was stirred at 0 °C to rt under Ar for 2.5 h. After 2.5 h, the reaction was quenched by water (10 mL) and extracted with ethyl

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acetate (10 mL × 2), washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography (*n*-hexane: ethyl acetate =2:1 (240 mL) to 1:1 (200 mL) to 1:2 (140 mL) to yield **54b** (136.8 mg, 0.5738 mmol, 71%).

^{1}H NMR ($CDCl_3$, 400 MHz): 7.067−7.028 (m, 1H), 6.753−6.711(m, 2H), 6.589−6.564 (m, 1H), 5.836 (bs, 1H), 4.584 (s, 2H), 0.835 (s, 9H), 0.000 (s, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz): 155.67, 143.06, 129.50, 118.41, 114.08, 113.22, 64.92, 26.02, 18.50, −5.19. HRMS (ESI-TOF $[M-H]^-$): Calcd for $C_{13}H_{21}O_2Si^-$ 237.1316. Found 237.1343. Anal. Calcd for $C_{13}H_{22}O_2Si + 0.1H_2O$; C, 65.05; H, 9.26; N, 0.00. Found C, 64.96; H, 9.03; N, 0.00.

*4-(((tert-Butyldimethylsilyl)oxy)methyl)phenol (**54c**).* Imidazole (258.8 mg, 3.8014 mmol) was added to the solution of 4-hydroxybenzyl alcohol **53c** (214.5 mg, 1.7279 mmol) in THF (4 mL) and TBSCl (286.5 mg, 1.9007 mmol) in THF (2 mL) was added to the reaction mixture at 0 °C under Ar. The whole was stirred at 0 °C for 10 min, and stirred at room temperature for 2 h. After 2 h, the reaction mixture was quenched by water (7 mL) and the whole was extracted with ethyl acetate (7 mL × 3), washed with brine, dried over $MgSO_4$ and evaporated under vacuum. The residue was purified by column chromatography (*n*-hexane: ethyl acetate =4:1 to ethyl acetate) to yield **54c** (357.0 mg, 1.4975 mmol, colorless oil, 87%, sm recovery 7.9 mg).

^{1}H NMR ($CDCl_3$, 400 MHz): 7.191−7.169 (2H, m), 6.794−6.759 (2H, m), 6.363 (1H, brs), 4.682 (2H, s), 0.952 (9H, s), 0.118 (6H, s). ^{13}C NMR ($CDCl_3$, 100 MHz): 154.95, 133.03, 127.98, 115.25, 65.02, 26.02, 18.49, −5.12. HRMS (ESI-TOF $[M + Na]^+$): Calcd for $C_{13}H_{22}NaO_2Si^+$: 261.1281. Found 261.1282. Anal. Calcd for $C_{13}H_{22}O_2Si$; C, 65.50; H,9.30; N, 0.00. Found C, 65.39; H, 9.25; N, 0.00.

*tert-Butyl O-(tert-butoxyl)3-(((tert-butyldimethylsilyl)oxy)-methyl)phenoxy)phosphoryl)-N-(tert-butoxylcarbonyl)-L-serinate) (**55b**).* Compound **40** (258.56 mg, 0.5726 mmol) and **54b** (105.0 mg, 0.4404 mmol) was dissolved in CH_2Cl_2 (1 mL) and toluene (0.1 mL) and coevaporated. The residue was dissolved in CH_2Cl_2 (1 mL) and *1H*-tetrazole (77.1 mg, 1.1011 mmol) in THF (1 mL) was added to the solution above. The whole was stirred at room temperature under Ar atmosphere for 20 h. After 20 h, the reaction mixture was quenched by saturated $NaHCO_3$ (aq) (10 mL) and extracted with CH_2Cl_2 (8 mL × 3). Combined organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography (*n*-hexane: ethyl acetate =4:1 (200 mL)) to yield crude trivalent phosphodiester compound (246.0 mg, 0.4088 mmol, 93%, colorless oil).

The trivalent phosphodiester compound (245.9 mg, 0.4086 mmol) was dissolved in CH_2Cl_2 (2.5 mL) and added *tert*-butylhydroperoxide in decane (0.1634 mL). The reaction mixture was stirred at room temperature under Ar atmosphere for 1.5 h. After 1.5 h, solvent was removed under vacuum and the residue was purified by column chromatography (*n*-hexane: ethyl acetate =4:1 (200 mL) to 2:1(180 mL)) to yield **55b** (185.9 mg, 0.3009 mmol, 74%, colorless oil, 68% (2 steps)).

^{1}H NMR ($CDCl_3$, 400 MHz): 7.208−7.148 (m, 1H), 7.054−7.014 (m, 2H), 6.993−6.947 (m, 1H), 5.388−5.291 (m, 1H), 4.625 (s, 2H), 4.384−4.322 (m, 1H), 4.297−4.252 (m, 1H), 4.244−4.196 (m, 1H), 1.420−1.404 (m, 9H), 1.350−1.334 (m, 18H), 0.842 (s, 9H), 0.000 (s, 6H).

^{13}C NMR ($CDCl_3$, 100 MHz): 168.14, 155.15, 150.85, 150.78, 143.56, 143.55, 129.33, 129.31, 122.27, 118.28, 118.24, 117,51, 117.48, 117.46, 84.89, 84.81, 84.77, 82.68, 82.67, 79.84, 68.04, 67.98, 67.92, 64.33, 54.42, 54.37. 54.29, 29.76, 29.75, 29.72, 28.26, 27.88, 27.86, 15.89, 18.34, 18.01, −5.33. ^{31}P NMR ($CDCl_3$, 161 MHz): −11.15, −11.47. HRMS (ESI-TOF $[M + Na]^+$): Calcd for $C_{29}H_{52}NNaO_9PSi^+$ 640.3041. Found 640.3039. Anal. Calcd for $C_{29}H_{52}NO_9PSi$; C, 56.38; H, 8.48; N, 2.27. Found C, 56.14; H, 8.35; N, 2.28.

*tert-Butyl O-(tert-butoxy(4-(((tert-butyldimethylsilyl)oxy)methyl)-phenoxy)phosphoryl)-N-(tert-butoxycarbonyl)-L-serinate (**55c**).* Compound **40** (130.3 mg, 0.2885 mmol) and compound **54c** (52.9 mg, 0.2219 mmol) was dissolved in CH_2Cl_2 (1 mL) and toluene (0.1 mL) and coevaporated. The residue was dissolved in CH_2Cl_2 (0.5 mL) and *1H*-tetrazole (38.9 mg, 0.5547 mmol) in THF (0.6 mL) was added to the solution above. The whole was stirred at room temperature under Ar atmosphere for 20 h. After 20 h, the reaction mixture was quenched by water (7 mL) and extracted with CH_2Cl_2 (6 mL × 2). Combined organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography (*n*-hexane: ethyl acetate =4:1) to yield crude trivalent phosphodiester compound (141.8 mg, 0.2356 mmol, quatititative, colorless oil).

The trivalent phosphodiester compound (141.8 mg, 0.2356 mmol) was dissolved in CH_2Cl_2 (1.5 mL) and added *tert*-butylhydroperoxide in decane (0.0943 mL). The reaction mixture was stirred at room temperature under Ar atmosphere for 70 min. After 70 min, solvent was removed under vacuum and the residue was purified by column chromatography (*n*-hexane: ethyl acetate =4:1 to 2:1) to yield **55c** (114.2 mg, 0.1849 mmol, 78%, white solid, 83% (2 steps)).

^{1}H NMR ($CDCl_3$, 400 MHz): 7.253−7.224 (2H, m), 7.135−7.097 (2H, m), 5.452−5.372 (1H, m), 4.668 (2H, s), 4.452−4.269 (3H, m), 1.487−2.469 (9H, m), 1.422−1.408 (18H, m), 0.902 (9H, s), 0.058 (6H, s). ^{13}C NMR ($CDCl_3$, 100 MHz): 168.18, 155.16, 149.71, 149.63, 138.00, 137.99, 127.20, 119.74, 119.72, 119.70, 119,67, 84.90, 84.86, 84.82, 84.78, 82.74, 82.73, 79.89, 68.01, 67.95, 67.91, 64.32, 54.43, 54.39, 54.35, 54.30, 29.78, 29.77, 29.74, 29.73, 28.29, 27.90, 27.89, 25.90, 18.35, −5.29. ^{31}P NMR ($CDCl_3$, 161 MHz): −11.04, −11.35. HRMS (ESI-TOF $[M + Na]^+$): Calcd for $C_{29}H_{52}NNaO_9PSi^+$: 640.3041. Found 640.3016.

Anal. Calcd for $C_{29}H_{52}NO_9PSi$; C, 56.38; H, 8.48;N, 2.27. Found C, 56.14; H, 8.29; N, 2.22.

*2-Hydroxybenzyl 3-(2-(undecyloxy)phenyl)propanoate (**56a**).* DIC (204.2 mg, 1.6111 mmol) and DMAP (19.7 mg, 0.1611 mmol) were added to the solution of salicylic alcohol **53a** (100.0 mg, 0.8055 mmol) and acid derivative (B) (258.2 mg, 0.8055 mmol) in CH_2Cl_2 (2 mL) and stirred at room temperature under Ar for 2 h. After 2 h, the reaction mixture was quenched by water (10 mL) and the whole was extracted with CH_2Cl_2 (8 mL × 3). Combined organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography (*n*-hexane: ethyl acetate =20:1 (420 mL) to 10:1 (220 mL) to 4:1 (250 mL)) to yield **56a** (130.7 mg, 0.3064 mmol, 38%, colorless oil).

^{1}H NMR ($CDCl_3$, 400 MHz): 7.357−7.283 (m, 2H), 7.217 (dt, 1H, J = 8.0 Hz, 7.6 Hz, 1.6 Hz), 7.127 (dd, 1H, J = 7.6 Hz, 1.6 Hz), 7.011 (dd, 1H, J = 8.0 Hz, 1.2 Hz), 6.963 (dt, 1H, J = 7.6 Hz, 7.2 Hz, 1.2 Hz), 6.897−6.842 (m, 2H), 4.001 (t, 2H, J = 6.4 Hz), 3.025−2.987 (m, 2H), 2.758−2.720 (m, 2H) 1.882−1.812 (m, 2H), 1.557−1.485 (m, 2H), 1.387−1.355 (m, 14H), 0.966 (t, 3H, J = 6.8). ^{13}C NMR ($CDCl_3$, 100 MHz): 176.04, 156.94, 155.64, 132.15, 131.08, 129.99, 128.38, 127.72, 121.86, 120.52, 120.26, 117.84, 111.05, 67.79, 63.22, 34.20, 32.00, 29.70, 29.67, 29.43, 29.36, 26.23, 22.77, 14.20. HRMS (ESI-TOF $[M + Na]^+$): Calcd for $C_{27}H_{38}NaO_4^+$ 449.2662. Found 449.2635. Anal. Calcd for $C_{27}H_{38}O_4$; C, 76.02; H, 8.98; N, 0.00. Found C, 75.74; H, 8.82; N, 0.00.

*O-(tert-Butoxy)3-hydroxymethyl)phosphporyl-N-tert-butoxylcarbonyl)-L-serine (**56b**).* **55b** (185.4 mg, 0.3001 mmol) in THF (2 mL) was added HF-Py (168.7 μL) in pyridine (415.6 μL) and stirred at room temperature under Ar for 2 h. After 2 h, the reaction was quenched by water (10 mL), extracted with CH_2Cl_2 (10 mL × 3). Combined organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography (*n*-hexane: ethyl acetate =2:1 (150 mL) to 1:1 (180 mL) to 1:2 (120 mL) to yield **56b** (57.7 mg, 0.1146 mmol, 38%).

^{1}H NMR ($CDCl_3$, 400 MHz): 7.240−7.188 (m, 1H), 7.148 (m, 1H), 7.085−7.054 (m, 1H), 7.024−6.978 (m, 1H), 5.404−5.276 (m, 1H), 4.587 (s, 2H), 4.370−4.330 (m, 1H), 4.331−4.233 (m, 2H), 2.739 (bs, 1H), 1.546−1.422 (m, 9H), 1.372−1.322 (m, 18H). ^{13}C NMR ($CDCl_3$, 100 MHz): 168.31, 155.19, 150.92, 150.85, 143.42, 129.64, 129.59, 123.30, 123.23, 118.93, 118.43, 85.20, 82.98, 82.93, 80.08, 68.03, 64.41, 54.42, 54.32, 29.81, 29.78, 28.28, 27.91, 27.87. ^{31}P NMR ($CDCl_3$, 161 MHz): −11.30, −11.37. HRMS (ESI-TOF $[M + Na]^+$): Calcd for $C_{23}H_{36}NNaO_9P^+$ 526.2176. Found 526.2149. Anal.

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Calcd for $C_{19}H_{30}NO_9P$: C, 54.86; H, 7.61; N, 2.78. Found C, 54.68; H, 7.45 ; N, 2.78.

*tert-Butyl O-(tert-butoxy(4-(hydroxymethyl)phenoxy)-phosphoryl)-N-(tert-butoxy-carbonyl)-L-serinate (**56c**).* Compound *tert*-butyl *O*-(*tert*-butoxy(4-(((*tert*-butyldimethylsilyl)oxy)methyl)-phenoxy)- phosphoryl)-*N*-(*tert*-butoxycarbonyl)-*L*-serinate **54c** (107.2 mg, 0.1735 mmol) was dissolved in THF (1 mL) and hydrogen fluoride-pyridine complex (119.7 μL) in pyridine (294.7 μL) was added to the solution above. This reaction mixture was stirred at room temperature under Ar atmosphere for 100 min. After 100 min, the reaction mixture was quenched by water (10 mL). Water layer was extracted with CH_2Cl_2 (8 mLX2), combined organic layer was washed with brine, dried over $MgSO_4$ and solvent was evaporated. The residue was purified by column chromatography (*n*-hexane: ethyl acetate =2:1 to 1:4) to yield **55c** (67.3 mg, 0.1337 mmol, 77%, colorless oil).

^{1}H NMR ($CDCl_3$, 400 MHz): 7.293−7.266 (2H, m), 7.141−7.103 (2H, m), 5.462−5.388 (1H, m), 4.603 (2H, s), 4.447−4.266 (3H, m), 2.641 (1H, brs), 1.488−1.471 (9H, m), 1.426−1.380 (18H, m). ^{13}C NMR ($CDCl_3$, 100 MHz): 168.18, 155.20,1150.06, 129.99, 137.86, 128.20, 119.99, 119.94, 85.15, 85.09, 85.02, 82.84, 82.82, 80.00, 68.12, 68.07, 68.02, 64.35, 54.38, 54.34, 54.29, 30.89, 29.79, 29.78, 29.75, 29.73, 28.29, 27.91, 27.89. ^{31}P NMR ($CDCl_3$, 161 MHz): −11.18, −11.45. HRMS (ESI-TOF [M + Na]$^+$): Calcd for $C_{23}H_{38}NNaO_9P^+$: 526.2176. Found 526.2189. Anal. Calcd for $C_{23}H_{38}NO_9P$; C, 54.86; H,7.61; N, 2.78. Found C, 54.91; H, 7.42; N, 2.84.

*tert-Butyl O-(tert-butoxy(2-(((3-(2-(undecyloxy)phenyl)-propanoyl)oxy)methyl)phenoxy)-phosphoryl)-N-(tert-butoxycarbonyl)-L-serinate (**57a**).* Compound **40** (177.4 mg, 0.3928 mmol) and **56a** (128.9 mg, 0.3022 mmol) was dissolved in CH_2Cl_2 (2 mL) and toluene (0.2 mL) and coevaporated. The residue was dissolved in CH_2Cl_2 (1 mL) and *1H*-tetrazole (63.5 mg, 0.9065 mmol) in THF (1 mL) was added to the solution above. The whole was stirred at room temperature under Ar atmosphere for 12 h. After 12 h, the reaction mixture was quenched by saturated $NaHCO_3$ (aq) (10 mL) and extracted with CH_2Cl_2 (8 mL × 3). Combined organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography (*n*-hexane: ethyl acetate =4:1 to yield crude trivalent phosphodiester compound (200.2 mg, 0.2534 mmol, 84%, colorless oil).

The trivalent phosphodiester compound (200.1 mg, 0.2533 mmol) was dissolved in CH_2Cl_2 (2 mL) and added *tert*-butylhydroperoxide in decane (0.1013 mL). The reaction mixture was stirred at room temperature under Ar atmosphere for 1.5 h. After 1.5 h, solvent was removed under vacuum and the residue was purified by column chromatography (*n*-hexane: ethyl acetate =4:1 (200 mL) to 2:1(240 mL)) to yield **57a** (132.7 mg, 0.1671 mmol, 66%, colorless oil, 55% (2steps)).

^{1}H NMR ($CDCl_3$, 400 MHz): 7.306−7.169 (m, 3H), 7.102−7.027 (m, 3H), 6.767−6.726 (m, 2H), 5.458−5.320 (m, 1H), 5.144−5.135 (m, 2H), 4.408−4.201 (m, 3H), 3.886−3.853 (m, 2H), 2.916−2.877 (m, 2H), 2.633−2.594 (m, 2H), 1.744−1.674 (m, 2H), 1.433−1.416 (m, 9H), 1.371−1.345 (m, 19H), 1.255−1.155 (m, 15H), 0.799 (t, 3H, *J* = 6.8 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): 172.99, 172.98, 168.16, 168.13, 156.95, 155.18, 148.69, 148.63, 129.96, 129.54, 129.50, 129.22, 128.79, 127.52, 127.43, 127.36, 124.93, 120.19, 119.88, 110.98, 85.28, 85.21, 82.77, 79 90, 77.41, 77.09, 76.77, 68.20, 68.14, 67.73, 60.90, 54.45, 54.39, 54.31, 34.12, 31.90, 29.79, 29.75, 29.61, 29.58, 29.36, 29.34, 29.32, 28.29, 27.92, 27.88, 26.16, 26.13, 22.67, 14.12. ^{31}P NMR ($CDCl_3$, 161 MHz): −11.03, −11.34. HRMS (ESI-TOF [M + Na]$^+$): Calcd for $C_{43}H_{68}NNaO_{11}P^+$ 828.4422. Found 828.4421. Anal. Calcd for $C_{43}H_{68}NO_{11}P$ + 0.1CH_2Cl_2; C, 63.58; H, 8.38; N, 1.72. Found C, 63.80; H, 8.40; N, 1.64.

*tert-Butyl O-(tert-butoxy(3-(((3-(2-(undecyloxy)phenyl)-propanoyl)oxy)methyl)phenoxy)-phosphoryl)-N-(tert-butoxycarbonyl)-L-serinate (**57b**).* Compound **56b** (57.6 mg, 0.1144 mmol) and carboxylic acid derivative (B) (40.3 mg, 0.1258 mmol) in CH_2Cl_2 (1 mL) was added EDCI (26.3 mg, 0.1373 mmol) and DMAP (2.8 mg, 0.0229 mmol) and stirred at room temperature under Ar for 6 h. After 6 h, the reaction mixture was diluted with CH_2Cl_2 (8 mL) and quenched by water (10 mL). The whole was extracted with CH_2Cl_2 (8 mLX2), washed with brine, dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography (acetone: *n*-hexane =1:5/*n*-hexane: ethyl acetate =2:1) to yield **57b** (27.8 mg, 0.0346 mmol, 30%/sm recovery 6.2 mg, 15%/ mixture 1.9 mg). ^{1}H NMR ($CDCl_3$, 400 MHz): 7.247−7.206 (m, 1H), 7.115−7.019 (m, 5H), 6.783−6.736 (m, 2H), 5.400−5.304 (m, 1H), 5.013−5.008 (m, 2H), 4.410−4.208 (m, 3H), 3.894−3.861 (m, 2H), 2.910−2.871 (m, 2H), 2.628−2.590 (m, 2H), 1.771−1.680 (m, 2H), 1.449−1.433 (m, 9H), 1.374−1.361 (m, 20H), 1.308−1.191 (m, 14H), 0.824−0.789 (m, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): 173.08, 168.17, 156.96, 155.20, 150.90, 150.84, 138.07, 129.95, 129.75, 128.75, 127.55, 124.37, 120.18, 119.70, 119.65, 119.60, 111.0085.10, 82.81, 79.99, 68.07, 67.97, 67.76, 65.36, 54.39, 34.11, 31.91, 29.81, 29.77, 29.62, 29.59, 29.36, 29.34, 29.31, 28.30, 27.93, 27.90, 26.16, 26.13, 25.61, 22.68, 14.12. ^{31}P NMR ($CDCl_3$, 161 MHz): −11.20, −11.50. HRMS (ESI-TOF [M + Na]$^+$): Calcd for $C_{43}H_{68}NNaO_{11}P^+$ 828.4422. Found 828.4431. Anal. Calcd for $C_{43}H_{68}NO_{11}P$; C, 64.08; H, 8.50; N, 1.74. Found: C, 64.01; H, 8.44; N, 1.72.

*tert-Butyl O-(tert-butoxy(4-(((3-(2-(undecyloxy)phenyl)-propanoyl)oxy)methyl) phenoxy)phosphoryl)-N-(tert-butoxycarbonyl)-L-serinate (**57c**).* Compound **56c** (66.7 mg, 0.1325 mmol) and carboxylic acid (B) (55.1 mg, 0.1722 mmol) were dissolved in CH_2Cl_2 (0.8 mL) and EDCI (38.1 mg, 0.1987 mmol) and *N,N*-dimthylaminopyridine (8.1 mg, 0.0662 mmol) were added to the solution above. The reaction mixture was stirred at room temperature under Ar atmosphere for 1.5 h. After 1.5 h, the reaction mixture was quenched by water (7 mL). Water layer was extracted with CH_2Cl_2 (8 mL × 2), combined organic layer was washed with brine, dried over $MgSO_4$ and solvent was evaporated. The residue was purified by column chromatography (*n*-hexane: ethyl acetate =4:1 to 2:1) to yield **57c** (42.1 mg, 0.0552 mmol, 39%, colorless oil, 24.7 mg (mixture)).

^{1}H NMR ($CDCl_3$, 400 MHz): 7.271−7.242 (2H, m), 7.18407.098 (4H, m), 6.848−6.802 (2H, m), 5.460−5.384 (1H, m), 5.053 (2H, s), 4.478−4.299 (3H, m), 3.943 (2H, t, *J* = 6.4 Hz), 2.950 (2H, t, *J* = 7.6 Hz), 2.660 (2H, t, *J* = 7.6 Hz), 1.816−1.746 (2H, m), 1.520−1.503 (9H, m), 1.483−1.432 (20H, m), 1.354−1.260 (14H, m), 0.891−0.857 (3H, m). ^{13}C NMR ($CDCl_3$, 100 MHz): 173.15, 168.17, 156.97, 155.18, 150.66, 150.58, 132.75, 129.96, 129.60, 128.74, 127.56, 120.17, 120.07,10.04, 119.99, 111.00, 85.17, 82.83, 79.98, 68.10, 67.74, 65.34, 54.40, 43.16, 31.91, 29.82, 29.81, 29.78, 29.76, 29.61, 29.59, 29.35, 29.34, 29.31, 28.31, 27.93, 27.91, 26.23, 26.13, 22.68, 14.12. ^{31}P NMR ($CDCl_3$, 161 MHz): −11.14, −11.46. HRMS (ESI-TOF [M + Na]$^+$): Calcd for $C_{43}H_{68}NNaO_{11}P^+$: 828.4422. Found 828.4418. Anal. Calcd for $C_{43}H_{68}NNaO_{11}P$; C, 62.83; H, 8.50; N, 1.70. Found C, 62.93; H, 8.15; N, 1.73.

*(2R,3R)-2-(((tert-Butyldimethylsilyl)oxy)methyl)tetrahydro-2H-pyran-3-yl-3-(2-((3-(4-chlorophenoxy)benzyl)oxy)phenyl)-propanoate (**60c**).* Compound **39b** (61.5 mg, 0.2497 mmol) and **79b** (95.6 mg, 0.2497 mmol) were dissolved in CH_2Cl_2 (1 mL) and diisopropylcarbodiimide (41.0 mg, 0.3246 mmol) and *N,N*-dimthylaminopyridine (9.1 mg, 0.0749 mmol) were added to the solution above. The reaction mixture was stirred at room temperature under Ar atmosphere for 22 h. After 22 h, the reaction mixture was evaporated under vacuum to remove solvent. The residue was purified by column chromatography (*n*-hexane: ethyl acetate =10:1 (220 mL) to 8:1 (90 mL) to 4:1 (100 mL) to 2:1 (150 mL)) to yield **60c** (108.4 mg, 0.1773 mmol, 71%, colorless oil, and recovered starting material **39b**: 23.0 mg).

^{1}H NMR ($CDCl_3$, 400 MHz): 7.362 (t, 1H, *J* = 8.0 Hz), 7.316−7.276 (m, 2H), 7.221−7.183 (m, 2H), 7.168−7.144 (m, 1H), 7.061 (m, 1H), 6.978−6.846 (m, 5H), 5.076 (s, 2H), 4.995 (m, 1H), 4.007−3.969 (m, 1H), 3.620−3.526 (m, 2H), 3.516−3.446 (m, 2H), 3.034−2.995 (m, 2H), 2.751−2.625 (m, 2H), 2.049−1.995 (m, 1H), 1.816−1.699 (m, 1H), 1.681−1.597 (m, 1H), 1.353−1.319 (m, 1H), 0.866 (s, 9H), 0.016, −0.003 (m, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz): 172.66, 157.27, 156.35, 155.69, 139.55, 130.15, 130.10, 129.79, 129.14, 128.45, 127.57, 122.00, 121.58, 120.90, 120.32, 117.98, 117.32, 117.23, 111.59, 78.65, 69.27, 68.06, 66.70, 62.11, 34.25, 27.50, 26.18, 25.86, 20.86, 18.22, 17.89. HRMS (ESI-TOF [M + Na]$^+$): Calcd for $C_{34}H_{43}ClNaO_6Si^+$ 633.2410, Found 633.2418. Anal. Calcd for

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$C_{34}H_{43}ClO_6Si$; C, 66.81; H, 7.09; N, 0.00; Found C, 66.63; H, 7.05; N, 0.00.

*(2R,3R)-2-(((tert-Butyldimethylsilyl)oxy)methyl)tetrahydro-2H-pyran-3-yl- 3-(2-((3-(p-tolyloxy)benzyl)oxy)phenyl)propanoate (**60d**).* Compound **39b** (73.4 mg, 0.2979 mmol) and **79c** (108.0 mg, 0.2979 mmol) were dissolved in CH_2Cl_2 (1.3 mL) and diisopropylcarbodiimide (48.9 mg, 0.3872 mmol) and *N,N*-dimthylaminopyridine (10.9 mg, 0.0894 mmol) were added to the solution above. The reaction mixture was stirred at room temperature under Ar atmosphere for 24 h. After 24 h, the reaction mixture was evaporated under vacuum to remove solvent. The residue was purified by column chromatography (*n*-hexane: ethyl acetate =10:1 (220 mL) to 8:1 (90 mL) to 6:1 (70 mL) to 4:1 (100 mL) to 2:1 (120 mL)) to yield **60d** (116.2 mg, 0.1967 mmol, 71%, colorless oil) and the recovered starting material **39b** (18.8 mg).

^{1}H NMR ($CDCl_3$, 400 MHz): 7.336 (t, 1H, *J* = 8.0 Hz), 7.219−7.151 (m, 5H), 7.053−7.048 (m, 1H), 6.969−6.855 (m, 5H), 5.067 (s, 2H), 5.005 (m, 1H), 4.016−3.977 (m, 1H), 3.630−3.546 (m, 2H), 3.524−3.452 (m, 2H), 3.022 (t, 2H, *J* = 7.6 Hz), 2.762−2.634 (m, 2H), 2.357 (s, 3H), 2.059−2.006 (M, 1H), 1.813−1.707 (m, 1H), 1.687−1.602 (m, 1H), 1.358−1.325 (m, 1H), 0.878 (s, 9H), 0.028−0.009 (m, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz): 172.72, 158.21, 156.45, 154.44, 139.25, 133.13, 130.31, 130.13, 129.89, 129.14, 127.57, 121.19, 120.81, 119.40, 117.39, 116.63, 111.59, 78.66, 69.39, 68.07, 66.68, 62.12, 34.26, 27.51, 26.23, 25.87, 20.86, 20.75, 18.23. HRMS (ESI-TOF [M + Na]$^+$): Calcd for $C_{35}H_{46}NaO_6Si^+$ 613.2956. Found 613.2958. Anal. Calcd for $C_{35}H_{46}O_6Si$; C, 71.15; H, 7.85; N, 0.00; Found C, 70.86; H, 7.90; N, 0.00.

*(2R,3R)-2-(Hydroxymethyl)tetrahydro-2H-pyran-3-yl-3-(2-((3-(4-chlorophenoxy)benzyl) oxy)phenyl)propanoate (**63c**).* Compound **60c** (103.6 mg, 0.1695 mmol) was dissolved in THF (1 mL) and hydrogen fluoride-pyridine complex (100.5 μL) in pyridine (247.9 μL) was added to the solution above. This reaction mixture was stirred at room temperature under Ar atmosphere for 6.5 h. After 6.5 h, the reaction mixture was quenched by water (10 mL). Water layer was extracted with CH_2Cl_2 (10 mLX2), combined organic layer was washed with brine, dried over $MgSO_4$ and solvent was evaporated. The residue was purified by column chromatography (*n*-hexane: ethyl acetate =2:1 (180 mL) to 1:1 (100 mL) to 1:2 (180 mL)) to yield **63c** (84.0 mg, 0.1690 mmol, 99%, colorless oil).

^{1}H NMR ($CDCl_3$, 400 MHz): 7.359 (t, 1H, *J* = 8.0 Hz), 7.309−7.259 (m, 2H), 7.206−7.153 (m, 3H), 7.069−7.064 (m, 1H), 6.973−6.852 (m, 5H), 5.067 (s, 2H), 4.944−4.936 (m, 1H), 4.027−3.987 (m, 1H), 3.532−3.461 (m, 3H), 3.314−3.290 (m, 1H), 3.003 (t, 2H, *J* = 7.6 Hz), 2.704 (t, 2H, *J* = 8.0 Hz), 2.474 (bs, 1H), 1.946−1.791 (m, 2H), 1.724−1.634 (m, 1H), 1.416−1.366 (m, 1H). ^{13}C NMR ($CDCl_3$, 400 MHz): 173.68, 157.28, 156.35, 155.66, 139.49, 130.15, 130.10, 129.80, 128.83, 128.46, 127.73, 122.00, 120.91, 120.33, 118.00, 117.22, 111.63, 78.51, 69.26, 68.06, 67.06, 61.9834.18, 27.82, 26.24, 20.84. HRMS (ESI-TOF [M + Na]$^+$): Calcd for $C_{28}H_{29}FNaO_6^+$ 519.1545, Found 519.1534. Anal. Calcd for $C_{28}H_{29}ClO_6 + 0.25CH_2Cl_2$; C, 65.57; H, 5.71; N, 0.00. Found C, 65.57; H, 5.83; N, 0.00.

*(2R,3R)-2-(Hydroxymethyl)tetrahydro-2H-pyran-3-yl- 3-(2-((3-(p-tolyloxy)benzyl)oxy)phenyl)propanoate (**63d**).* Compound **60d** (110.9 mg, 0.1877 mmol) was dissolved in THF (1.) and hydrogen fluoride-pyridine complex (110.7 μL) in pyridine (274.5 μL) was added to the solution above. This reaction mixture was stirred at room temperature under Ar atmosphere for 19 h. After 19 h, the reaction mixture was quenched by water (10 mL). Water layer was extracted with CH_2Cl_2 (10 mLX2), combined organic layer was washed with brine, dried over $MgSO_4$ and solvent was evaporated. The residue was purified by column chromatography (*n*-hexane: ethyl acetate =2:1 (180 mL) to 1:1 (120 mL) to 1:2 (120 mL)) to yield **63d** (57.0 mg, 0.1196 mmol, 64%, colorless oil/29.2 mg (small amount of impurity was remained), 0.0613 mmol, 33%, colorless oil).

^{1}H NMR ($CDCl_3$, 400 MHz): 7.349−7.310 (m, 1H), 7.201−7.142 (m, 5H), 7.056−7,051 (m, 1H), 6.959−6.862 (m, 5H), 5.058 (s, 2H), 4.951−4.944 (m, 1H), 4.033−3.994 (m, 1H), 3.574−3.465 (m, 3H), 3.349−3.264 (m, 1H), 3.007 (t, 2H, *J* = 7.6 Hz), 2.710 (t, 2H, *J* = 7.6 Hz), 2.447 (bs, 1H), 2.347 (s, 3H), 1.952−1.799 (m, 2H), 1.727−1.638 (m, 1H), 1.423−1.370 (m, 1H).

^{13}C NMR ($CDCl_3$, 400 MHz): 173.76, 158.22, 156.45, 154.41, 139.19, 133.18, 130.32, 130.14, 129.87, 128.84, 127.72, 121.19, 120.82, 119.39, 117.41, 116.65, 111.64, 78.50, 69.40, 68.07, 67.04, 61.97, 34.19, 27.83, 26.29, 20.84, 20.74. HRMS (ESI-TOF [M + Na]$^+$): Calcd for $C_{29}H_{32}NaO_6^+$ 499.2091. Found 499.2089. Anal. Calcd for $C_{29}H_{32}O_6 + 0.2CH_2Cl_2$; C, 71.10; H, 6.57; N, 0.00. Found C, 70.71; H, 6.76; N, 0.00.

*tert-Butyl O-(tert-butoxy(((2R,3R)-3-((3-(2-((3-(4-chlorophenoxy)-benzyl)oxy)phenyl)- propanoyl)oxy)tetrahydro-2H-pyran-2-yl)-methoxy)phosphoryl)-N-(tert-butoxycarbonyl)-L-serinate (**66c**).* Compound **40** (52.3 mg, 0.1158 mmol) and **63c** (86.3 mg, 0.1736 mmol) were dissolved in CH_2Cl_2 (1 mL) and toluene (0.1 mL) and coevaporated. The residue was dissolved in CH_2Cl_2 (1 mL) and 1H-tetrazole (16.2 mg, 0.2315 mmol) in THF (1 mL) was added to the above solution. The whole was stirred at room temperature under Ar atmosphere for 23 h. After 23 h, the reaction mixture was quenched by saturated aqueous $NaHCO_3$ (7 mL) and extracted with CH_2Cl_2 (8 mLX2). Combined organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography (*n*-hexane: ethyl acetate =4:1 (150 mL) to 2:1 (120 mL) to 1:1 (140 mL)) to yield crude trivalent phosphodiester compound (37.5 mg, 0.0438 mmol, 38%, colorless oil).

The trivalent phosphodiester compound (37.4 mg, 0.0437 mmol) was dissolved in CH_2Cl_2 (0.8 mL) and added *tert*-butylhydroperoxide in decane (0.0873 mL). The reaction mixture was stirred at room temperature under Ar atmosphere for 1 h. After 1 h, solvent was removed under vacuum and the residue was purified by column chromatography (*n*-hexane: ethyl acetate =2:1 (270 mL) to 1:1 (160 mL)) to yield **66c** (30.5 mg, 0.0350 mmol, 80%, colorless oil, 30% (2steps)).

^{1}H NMR ($CDCl_3$, 400 MHz): 7.287 (t, 1H, *J* = 8.0 Hz), 7.236−7.192 (m, 2H), 7.136−7.064 (m, 3H), 6.972 (m, 1H), 6.895−6.835 (m, 3H), 6.816−6.767 (m, 2H), 5.483−5.464 (m, 1H), 5.005 (s, 2H), 4.829−4.823 (m, 1H), 4.282−4.207 (m, 2H), 4.156−4.108 (m, 1H), 3.943−3.897 (m, 1H), 3.877−3.785 (m, 2H), 3.638−3.582 (m, 1H), 3.432−3.364 (m, 1H), 2.939−2.862 (m, 2H), 2.671−2.540 (m, 2H), 1.900−1.865 (m, 1H), 1.722−1.524 (m, 2H), 1.391−1.366 (m, 27H), 1.281−1.230 (m, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): 172.63, 168.39, 157.24, 156.31, 155.66, 155.32, 139.53, 130.10, 129.77, 128.92, 128.43, 127.61, 121.96, 120.89, 129.30, 117.94, 117.18, 111.62, 83.93, 82.60, 82.58, 78.88, 76.50, 69.26, 67.82, 67.75, 67.52, 66.84, 66.74, 54.41, 34.10, 29.79, 29.75, 29.71, 28.33, 27.95, 27,48, 27.42, 26.11, 20.47. ^{31}P NMR ($CDCl_3$, 161 MHz): −5.50, −5.78 HRMS (ESI-TOF [M + Na]$^+$): Calcd for $C_{44}H_{59}ClNNaO_{13}P^+$ 898.3305, Found 898.3312. Anal. Calcd for $C_{44}H_{59}ClNO_{13}P + 0.6CH_2Cl_2$; C, 57.83; H, 6.51; N, 1.51. Found: C, 57.80; H, 6.49; N, 1.57.

*tert-Butyl O-(tert-butoxy(((2R,3R)-3-((3-(2-((3-(p-tolyloxy)benzyl)-oxy)phenyl)-propanoyl)oxy)tetrahydro-2H-pyran-2-yl)methoxy)-phosphoryl)-N-(tert-butoxycarbonyl)-L-serinate (**66d**).* Compound **40** (46.5 mg, 0.1030 mmol) and **63d** (73.6 mg, 0.1545 mmol) was dissolved in CH_2Cl_2 (1 mL) and toluene (0.1 mL) and coevaporated. The residue was dissolved in CH_2Cl_2 (0.5 mL) and 1H-tetrazole(14.4 mg, 0.2059 mmol) in THF (1 mL) was added to the solution above. The whole was stirred at room temperature under Ar atmosphere for 24 h. After 24 h, the reaction mixture was quenched by saturated $NaHCO_3$ (aq) (6 mL) and extracted with CH_2Cl_2 (8 mL × 3). Combined organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography (hexane: ethyl acetate =4:1 (150 mL) to 2:1 (150 mL) to 1:1 (160 mL)) to yield crude trivalent phosphodiester compound (17.7 mg, 0.0212 mmol, 21%, colorless oil).

The trivalent phosphodiester compound (17.7 mg, 0.0212 mmol) was dissolved in CH_2Cl_2 (0.4 mL) and added *tert*-butylhydroperoxide in decane (0.0423 mL). The reaction mixture was stirred at room temperature under Ar atmosphere for 1.5 h. After 1.5 h, solvent was removed under vacuum and the residue was purified by column chromatography (hexane:ethyl acetate =2:1 (180 mL) to 1:1 (200

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mL) to 1:2 (120 mL)) to yield **66d** (15.5 mg, 0.0182 mmol, 86%, colorless oil, 29%(2steps)).

^{1}H NMR ($CDCl_3$, 400 MHz): 7.316 (t, 1H, J = 8.0 Hz), 7.177−7.130 (m, 5H), 7.018 (m, 1H), 6.939−6.837 (m, 5H), 5.553−5.524 (m, 1H), 5.057 (s, 2H), 4.901−4.895 (m, 1H), 4.352−4.225 (m, 2H), 4.215−4.177 (m, 1H), 4.011−3.964 (m, 1H), 3.945−3.854 (m, 2H), 3.706−3.663 (m, 1H), 3.497−3.430 (m, 1H), 3.022−2.930 (m, 2H), 2.743−2.608 (m, 2H), 2.336 (s, 3H), 1.970−1.936 (m, 1H), 1.791−1.593 (m, 2H), 1.560−1.436 (m, 27H), 1.387−1.274 (m, 1H).

^{13}C NMR ($CDCl_3$, 100 MHz): 172.67, 168.40, 158.16, 156.40, 155.22, 154.40, 139.23, 133.14, 130.29, 130.07, 129.93, 127.60, 121.14, 120.80, 119.35, 117.35, 116.59, 111.62, 82.60, 82.57, 79.89, 76.50, 69.38, 67.82, 67.75, 67.51, 66.82, 66.71, 66.45, 54.42, 34.10, 29.79, 29.75, 29.71, 28.33, 27.95, 27.49, 27.43, 26.15, 20.72, 20.47. ^{31}P NMR ($CDCl_3$, 161 MHz): δ −5.50, −5.79. HRMS (ESI-TOF [M + Na]$^+$): Calcd for $C_{45}H_{62}NNaO_{13}P^+$ 878.3851. Found 878.3847. Anal. Calcd for $C_{45}H_{62}NO_{13}P$ + 1.2H_2O; C, 61.59 ; H, 7.40 ; N, 1.60. Found C, 61.22; H, 7.05; N, 1.58.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.5b01925.

Details of synthesis of nonaromatic LysoPS analogues and that of aromatic LysoPS analogues, and Figures S1−S8. (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*Design and Chemical study: For T.O.: phone, +81-3-5841-4730; fax, +81-3-5841-4735; E-mail, ohwada@mol.f.u-tokyo.ac.jp.

*Biological study: For J.A.: phone, +81-22-795-6860; fax, +81-22-795-6859; E-mail, jaoki@m.tohoku.ac.jp. Tohoku University.

Author Contributions

S.J. and A.I. contributed equally to this work. S. J., S. N., M. S., M. I., Y. O. and T. O. (The University of Tokyo) performed chemical studies including design and synthesis of compounds. S. J. studied solution structures of the model compounds and performed calculational studies. A. I., A. U., T. K., T. K., K. K., K. M. and J. A. (Tohoku University) performed biological studies including TGFα shedding assay, Ca^{2+} assay, and actin stress fiber formation assay.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

LysoPS, lysophosphatidylserine; LPS, lysophosphatidylserine receptor; PS, phosphatidylserine; TGFα, transforming growth factor-alpha; AP-TGFα, alkaline phosphatase-tagged TGFα; HBSS, Hanks' balanced salt solution; HEPES, 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid; CM, conditioned media; DMEM, Dulbecco's modified Eagle's medium; DAPI, 4′,6-diamidino-2-phenylindole; Mp, melting point; EDCI, *N*-(3-(dimethylamino)propyl)-*N*-ethylcarbodiimide hydrochloride; HOBt·H_2O, 1-hydroxybenzotriazole hydrate; TBAI, tetrabutylammonium iodide; DIPEA, *N*,*N*-diisopropylethylamine

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