博士論文

論文題目 Synthesis of Conformationally Constrained Lysophosphatidylserine and Elucidation of Their Bioactive Structures against Specific GPCRs

（配座を固定したリゾホスファチジルセリンの合成と特異的なG−タンパク質共役型受容体に対する活性構造の探索）

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ジョン　セジン

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Chapter 1. Introduction

1-1. General Interests of G-Protein Coupled Receptors (GPCRs)

G protein-coupled receptors (GPCRs) are considerably important family of proteins, which constitute a large superfamily of cell surface receptors. GPCRs mediate external cellular signal to transfer into internal G-protein and send the message for appropriate cellular and physiological response. These processes are extremely intricate and different stimulators induce different wide range of responses. This huge protein family is frequently the drug target in the pharmaceutical industry due to its involvement in every organ system and participation in variety of disease, such as cancer, diabetes, inflammation and central nervous system disorders, etc. According to the recent report, approximately 30% of prescription drugs address GPCRs[1].

These miscellaneous GPCRs have been studied in many different fields, deorphanizing of unknown GPCRs using high throughput screening, genomic analysis to determine expression in specific organs, identification of structure of GPCR by crystallization, which is challenging work due to its intrinsic variety, computational homology modeling to determining three-dimensional structure and structure activity relationship studies etc.

Since identification of bovin rhodopsin crystal structure in 2000[2], human β2 adrenergic receptor in 2007[3], β2 adrenergic receptor-Gs ternary complex with high-resolution[4] was reported in 2011. Continuously, dopamine D3 receptor[5], chemokine receptor CXCR4[6] were reported. Recently, crystal structure of sphingosine-1-phosphate (S1P) receptor[7], human protease-activated receptor 1[8], P2Y12 receptor in complex with antithrombotic drug[9], and agonist-bound human P2Y12[10] were reported. Even though many GPCRs crystal structures have been clarified with development of computational technology, it is still a challenging work to elucidate three-dimensional structures because of difficult purification and crystallization.

1-2. General Functions of G-Protein Coupled Receptors (GPCRs)

G-protein coupled receptors (GPCRs), also known as seven transmembrane domain receptors, have been studied over the decades. GPCRs are proteins located in the plasma membrane and mediate a wide range of physiological signals from the outside of the cell. GPCRs consist of extracellular N-terminus, seven transmembrane α-helices connected by three intracellular loops and three extracellular loops, finally an intracellular C-terminus. Signaling transduction is initiated by binding of ligands including hormones, pheromones, neurotransmitters, peptides, ions and lipids etc. to the ligand-binding domain and results into appropriate cellular and physiological responses through the conformational change of receptors to activate G proteins, which is heterotrimer of significant components, G_{α} and G_{β}/G_{γ} subunits. Activation of receptors induces exchange of GDP for GTP on the Gα subunit of the heterotrimeric complex and dissociation of G_{β}/G_{γ} dimer from G_{α}. These moieties then become free to act upon their downstream effectors and thereby initiate unique intracellular signaling responses (Figure 1).

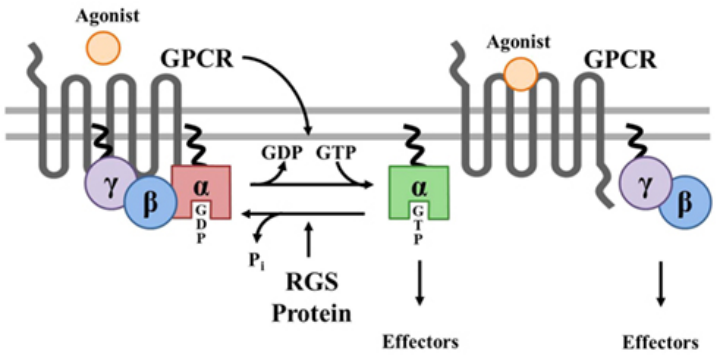


Figure 1. Regulation of GPCRs signaling for agonist binding case [11]

(RGS: regulator of G-protein signaling)

The receptor proteins in the membrane are dynamic and can take various conformations depending on the ligands and induce specific biological events. Therefore, GPCRs are involved in many kinds of diseases and the targets of approximately 50% of all modern medicinal drugs.

1-3. Generation of Endogenous Lipid Ligand of GPCRs: Lysophosphatidylserine

Phospholipids (PLs) are major component of all cell membranes and consist of hydrophilic head, which is able to ionize at pH 7, and hydrophobic tail groups of long acyl chains that are repelled by water and forced to aggregate. Consequently, these specific properties allow phospholipids to be amphipathic and to form lipid bilayer.

Lysophospholipids (LPLs) refer to the phospholipid that is lost one of its two acyl chains and several kinds of LPLs are known such as Lysophosphatidic acid (LPA), Lysophosphaticylcholine (LPC), Lysophosphatidylethanolamine (LPE), Lysophosphatidylinositol (LPI), Lysophosphatidylserine (LPS, LysoPS) and sphingosine-1-phosphate (S1P) (Figure 2). These are not only structural components of cellular membrane but also biologically active molecules interacting with GPCRs, influencing a wide variety of processes such as immunity, vascular development, neurogenesis and carcinogenesis etc.

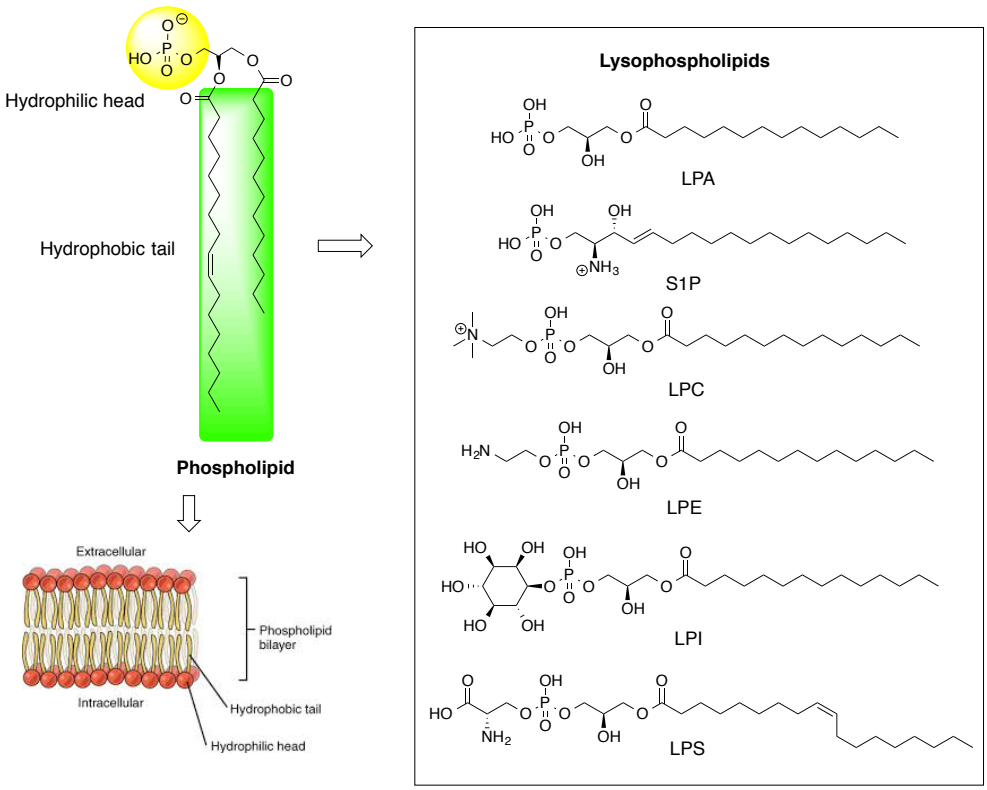


Figure 2. Formation of lipid bilayers and several kinds of Lysophospholipids

Phosphatidylserine (PS) is one of the phospholipids, usually kept on the inner-leaflet of cell membranes, and becomes exposed on the surface of the cell when a cell undergoes apoptosis. PS is relatively minor constituent of most biological membranes. However, the low abundance of PS is out-weighted by its physiological and biochemical properties. PS contains *L*-serine, which bears three ionizable groups, i.e., phosphate moiety, the amino group and the carboxyl function of *L*-serine and resulted in anionic feature of total charge of PS.

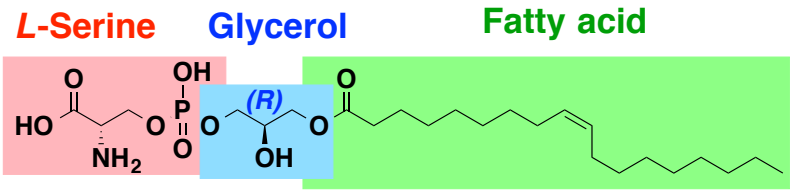


Figure 3. Structure of lysophsphatidylserine (lysoPS, LPS)

Lysophosphatidylserine (LysoPS) is derived from phosphatidylserine (PS) by hydrolysis of one acyl chain (Figure 3). LysoPS has a modular structure containing single fatty acid, *L*-serine and glycerol connected by phosphodiester and ester linkage.

The acyl chain on sn-1 position of PS can be hydrolyzed by PS-specific lipase A_1 (PS-PLA_1), which is secreted from activated platelets to produce 2-acyl-lysoPS containing the acyl chain at sn-2 position of glycerol backbone. The acyl chain on sn-2 position is also hydrolyzed by non-specific secretory type II phospholipase A_2 (s-PLA_2-IIA) to afford 1-acyl-lysoPS, which has the acyl chain on sn-1 position. However, mutual transformation of lysophopholipids via acyl migration from sn-2 to sn-1 position can occur and its equilibrium is inclined to more stable 1-acyl-lysoPS with the ratio of 9:1[12] (Figure 4).

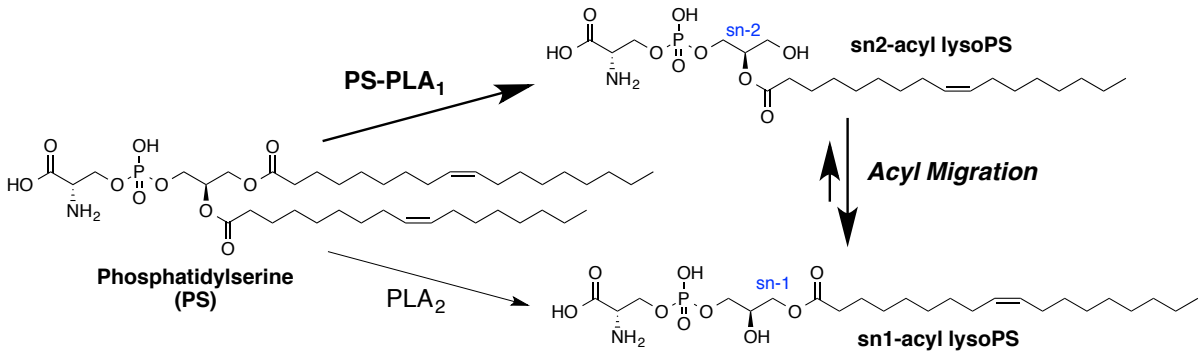


Figure 4. Generation of lysophosphatidylserine (LysoPS) from phosphatidylserine (PS) and mutual transformation via acyl migration

There are several physiological activities of lyso PS.

1) Stimulation of degranulation on rat peritoneal mast cell (RPMCs)[13]

2) Inhibition of mitogen-induced T cell activation[14]

3) Potentiation of nerve growth factor-induced differentiation of PC12 cells[15]

4) Induction of a transient increase in cytosolic free calcium [Ca^{2+}] in Jurkat T cells[16]

5) Stimulation of chemotactic migration in U87 human glioma cells[17, 18]

1-4. LysoPS and its specific receptors

Our collaborators identified the lysoPS-specific GPCRs, P2Y10 (LPS2), A630033H20 (LPS2-like) and GPR174 (LPS3), which are coupled with $G\alpha_{12/13}$, during the screening to find ligands of orphan GPCRs[19]. Human A630033H20 is the product of a truncated nonfunctional pseudo gene, but homologs of P2Y10 and GPR174 have been found in human, mouse, and rat, and they respond to endogenous lysoPS with EC_{50} values of submicromolar concentration. In addition, together with GPR34, which was identified as lysoPS-specific receptor by T. Sugo in 2006[20] during a ligand fishing study, P2Y10 and GPR174 are phylogenetically close from each other (Figure 5). Therefore, we focused on these three receptors, P2Y10, GPR174 and GPR34 in this study.

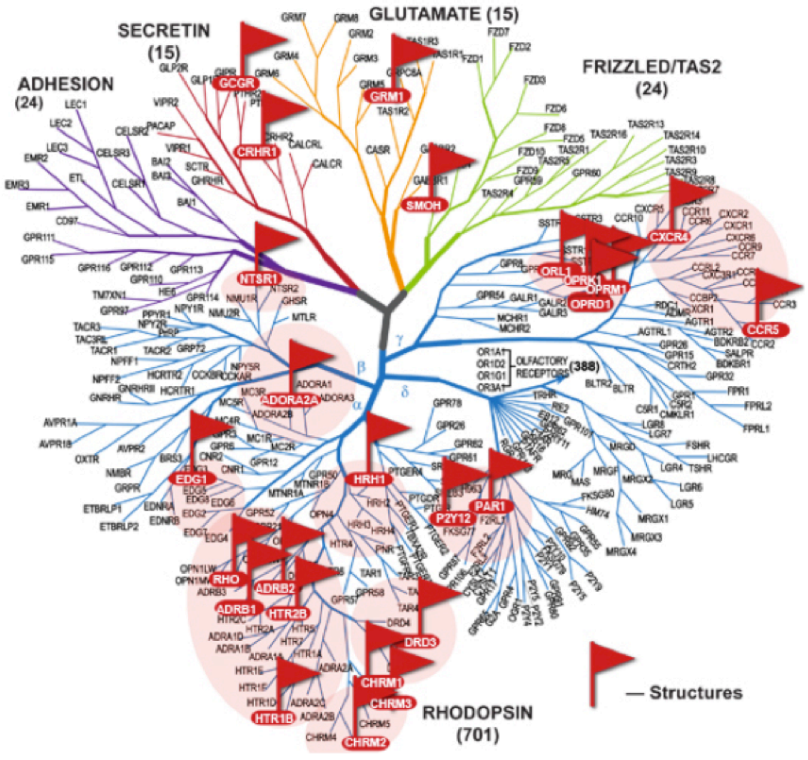


Figure 5. Phylogenetic tree of GPCRs [21]

According to the research results so far, these receptors are expressed in immune related tissues and involved in the immunological challenges. Liebscher et al. reported that GPR34 has an important role in immune system[22] suggested from the study of GPR34-deficient mice and P2Y10 is involved in biological activity of a T lymphocyte[23]. In addition, recent genome-wide association studies (GWAS) suggested that higher levels of GPR174 expression is associated with Graves' disease[24], which is female preponderant autoimmune illness. Therefore, interrelationship between lysoPS and these receptors, and association of immune system are critical research subject in the field of pharmaceutical sciences. To prove the interaction of ligand, lysoPS as a immunological modulator, with its specific receptors, two possible methods, ligand-based and structure-based approaches, could be applied. However, identification of X-ray crystal structures of the relevant GPCRs is still challenging work due to their high intrinsic flexibility.

1-5. Biological Assay: TGFα shedding assay

All lysoPS analogues synthesized in this study were evaluated their agonistic activities using the TGFα shedding assay which is developed by our collaborators in Tohoku university.

They reported that ectodomain shedding of TGFα occurred downstream of the lysophosphatidic acid (LPA) receptor P2Y5 signaling and this event is responsible for LPS-induced hair follicle development[25]. In that study, they found that LPA-induced ectodomain shedding of AP-TGFα enzyme (TACE, also known as ADAM17) by reconstitution of LPS-induced TGFα shedding in HEK293 cells. This prompted to lead the idea of using ectodomain shedding of AP-TGFα to detect other GPCRs, especially $G_{12/13}$-coupled GPCRs because AP-TGFα shedding occurrs almost downstream of $G_{12/13}$ and G_q signaling.

The principles of the TGFα shedding assay are GPCR-induced ectodomain shedding of membrane-bound pro-AP-TGFα, a reporter enzyme, and quantification of the released AP-TGFα in the conditioned medium (Figure 6).

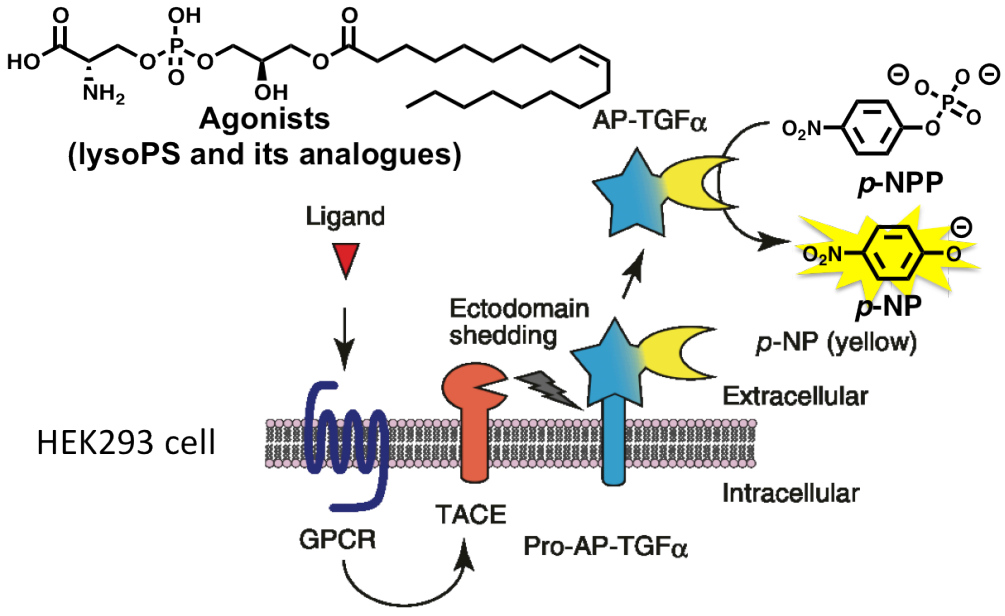


Figure 6. TGFα-shedding assay [19]

The procedure of the TGFα shedding assay is as follows (Figure 7).

① Transfection with an expression plasmid encoding AP-TGFα with or without a GPCR-encoding plasmid.

② Reseeding of the transfected cells in serum-free medium in a 96-well plate.

③ Stimulation with a GPCR ligand for 1h, which results in accumulation of released AP-TGFα in the conditioned medium.

④ Separation and transfer of the conditioned medium.

⑤ Addition of para-nitrophenylphosphate (*p*-NPP), a substrate for alkaline phosphatase, to both the conditioned medium and the remaining cells.

⑥ Measurement of para-nitrophenol (*p*-NP) production with an optical plate reader at a wavelength of 405 nm (OD_{405}) before incubation (background) and after a 1h incubation.

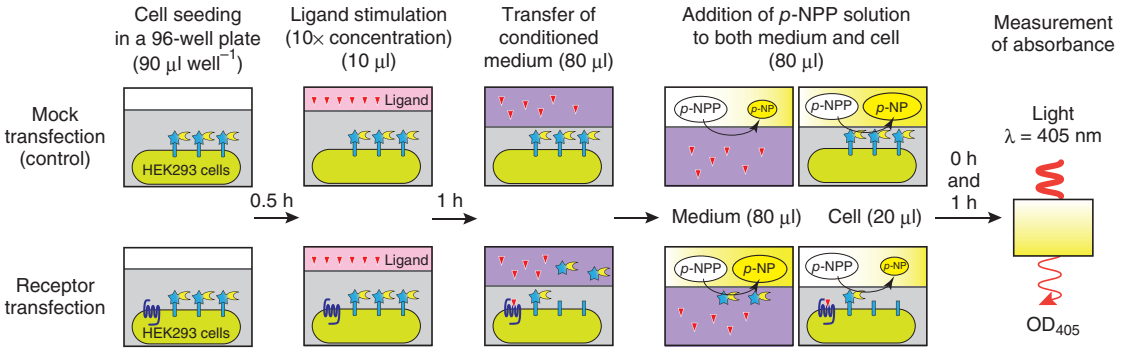


Figure 7. Protocol of the TGFα shedding assay [19]

The results of evaluated lysoPS analogues are represented with '+' symbol on the basis of '+++' agonistic activity of lysoPS as standard in addition to EC_{50} values. '+' means 10-fold potency to lysoPS. To give an example, if the agonistic activity is 10-fold more potent than lysoPS, it is represented '++++'.

Chapter 2. Aims and Strategies of This Study

2-1. Our Previous Studies about the Effect of Modifications of LysoPS

We have done research on structure-activity relationship with various modifications of lysoPS to obtain information of structural requirement for activation of each receptor and tried to find structural features crucial for ligand potency and selectivity (Figure 8).

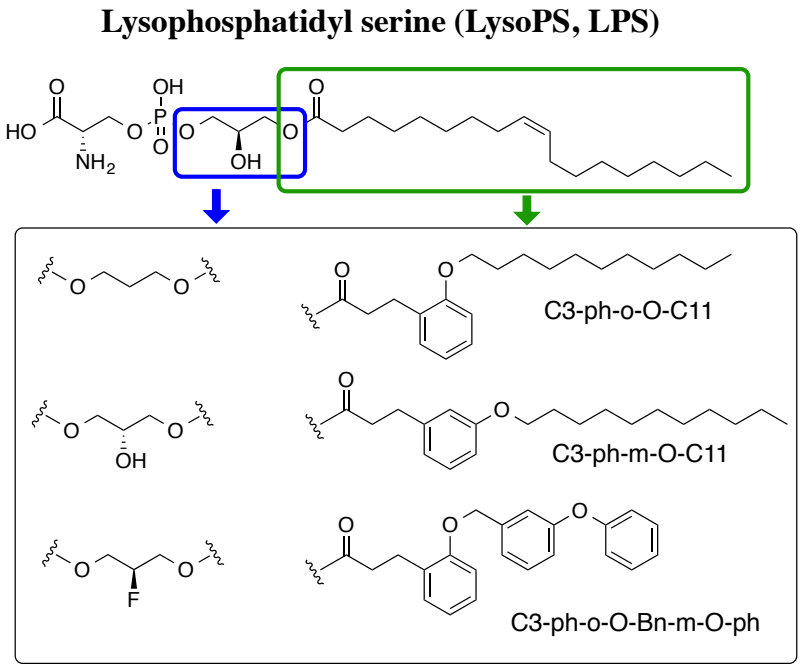


Figure 8. Structure activity relationship studies of lysoPS to find out the structural requirement for activation of three receptors

Especially, for fatty acid chain modification, we tried to aromatize the long hydrocarbon acyl chain by introducing benzene ring(s), which leads to less flexible lysoPS analogue. From this study, it is disclosed that modification of fatty acid moiety showed great impacts on activity and selectivity.

The analogue containing the acyl chain, C3-ph-o-O-C11 (Figure 8), expressed potent agonistic activity toward three receptors. Especially, it is the most potent against P2Y10 (Figure 9). In the case of the analogue bearing the acyl chain, C3-ph-m-O-C11 (Figure 8), has strong GPR174 activity including the activity toward P2Y10 (Figure 9). In addition, lysoPS analogue containing the acyl chain, C3-ph-o-O-Bn-m-O-ph (Figure 8), expressed potent GPR34 activity together with P2Y10 activity similar to that of lysoPS (Figure 9).

In other words, we could say lysoPS analogue-C3-ph-o-O-C11 is a pan-agonist, lysoPS analogue-C3-ph-m-O-C11 is a dual agonist for P2Y10 and GPR174, and lysoPS analogue-C3-ph-o-O-Bn-m-O-ph is a dual agonist for GPR34 and P2Y10.

It was also revealed that the glycerol moiety is crucial for activity and selectivity. For example, the elimination of a hydroxyl group on sn-2 position of the glycerol leads to disappearance of GPR174 agonistic activity. In this context, active structures are sensitive to small structural modification.

I expected that it is possible to increase selectivity and potency by constraining other part of lysoPS in addition to aromatized fatty acid chains. Therefore, I chose the glycerol backbone for further restriction of freedom. When we use ring structures to mimic the glycerol moiety, we can define the stereochemistry and regiochemistry of the lysoPS mimics. Finally, I chose tetrahydropyran structures to mimic the glycerol moiety, having non-equivalent hydroxyl group instead of glycerol.

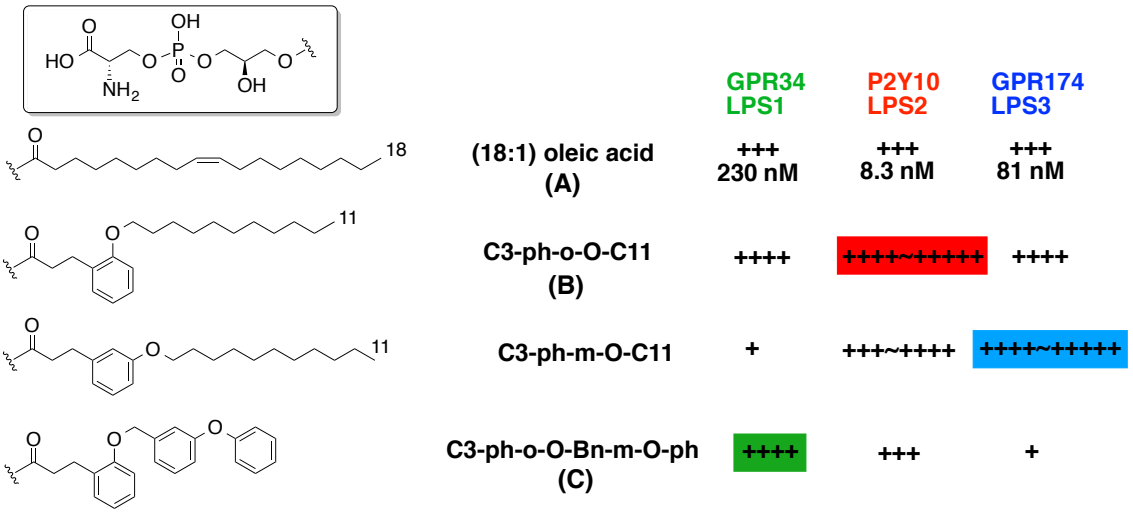


Figure 9. Representative fatty acids and shedding assay results of lysoPS analogues containing the acyl chains

In order to generate selective analogue for each receptor, acyl chain, C3-ph-o-O-C11 and C3-ph-o-O-Bn-m-O-ph including oleic acid were used for this study, because they have the strongest potency against each receptor, P2Y10 and GPR34 respectively.

The names of acyl chains will be abbreviated for convenience hereafter, (18:1) oleic acid is **A**, C3-ph-o-O-C11 is **B** and C3-ph-o-O-Bn-m-O-ph is **C** (Figure 9).

2-2. About What I Revealed in This Study

Lysophosphatydylserine (LysoPS) is a pan-agonist toward GPR34, P2Y10 and GPR174, and activates them simultaneously. This is because lysoPS is extremely flexible to take various conformations. At this point, it is interest to propose the active conformations of lysoPS toward individual receptors.

While we have known some information about the structural requirement of lysoPS for receptor activation, *three-dimensional structures of active conformations for each receptor* are still unknown. It is also unclear about a question whether *the active conformations are similar or different in the view of structures for individual receptors*, as shown in Figure 10. In order to shed light on the above issues, I tried to generate novel conformationally constrained lysoPS analogues and elucidate active conformations of lysoPS for each receptor activation.

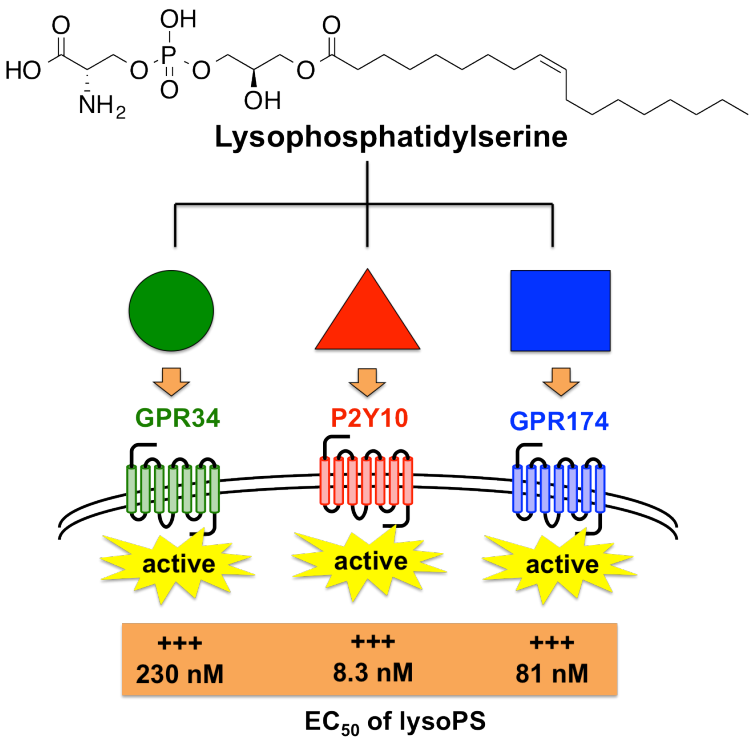


Figure 10. Assumption of different active conformations of lysoPS toward each receptor

In this study, the aims of my research are as follows:

(1) I execute structure modification to generate restricted conformation to suppress its flexibility, hopefully leading to potent and selective agonists to activate three subtype receptors, GPR34, P2Y10 and GPR174. Particularly in this study I used a tetrahydropyran structures to constrain the glycerol moiety. Thus I focused on GPR34 and P2Y10, not GPR174 because the sn-2 hydroxy group is embedded into the ring structure.
(2) I tried to elucidate active conformation of lysoPS for GPCRs activation, by using conformation-restricted derivative of lysoPS, and grafting biological activities onto computational study.

I first synthesized the conformationally constrained lysophosphatidylserine analogues by introducing a tetrahydropyran moiety, followed by evaluation of their biological activities, and conformational study by calculation process. As a fortunate result, we obtained selective and potent cyclic lysoPS analogues toward GPR34 and P2Y10 and suggest active conformation of lysoPS against each receptor, that is, a folded structure of lysoPS is responsible for GPR34 activation and extended glycerol backbone of lysoPS is significant for P2Y10 activation. Detailed methods and contents are introduced in the following chapters.

2-3. Design of the Conformationally Constrained LysoPS Analogues

It is revealed that LysoPS is composed of several substructures to form a modular structure in our previous SAR study and identified the several significant structural functions of LysoPS by modification of amino acid, glycerol and fatty acid chain including the ester linkage. Especially, the clear result what we noticed from this study is that the hydroxyl group on sn-2 position of glycerol backbone has an important role in activation of GRP174 as a hydogen bonding donor. Therefore, we introduced 2-hydroxymethyl-3-hydroxytetrahydropyran skeletone instead of glycerol moiety in order to increase the selectivity by elimination of the hydrogen of the hydroxyl group on glycerol backbone and expected to develop a single agonist for each receptor (Figure 11). Moreover, we predicted that constrained lysoPS molecule is more useful for conformation study because of reduced flexibility to suppress dynamic conformation change in some degree.

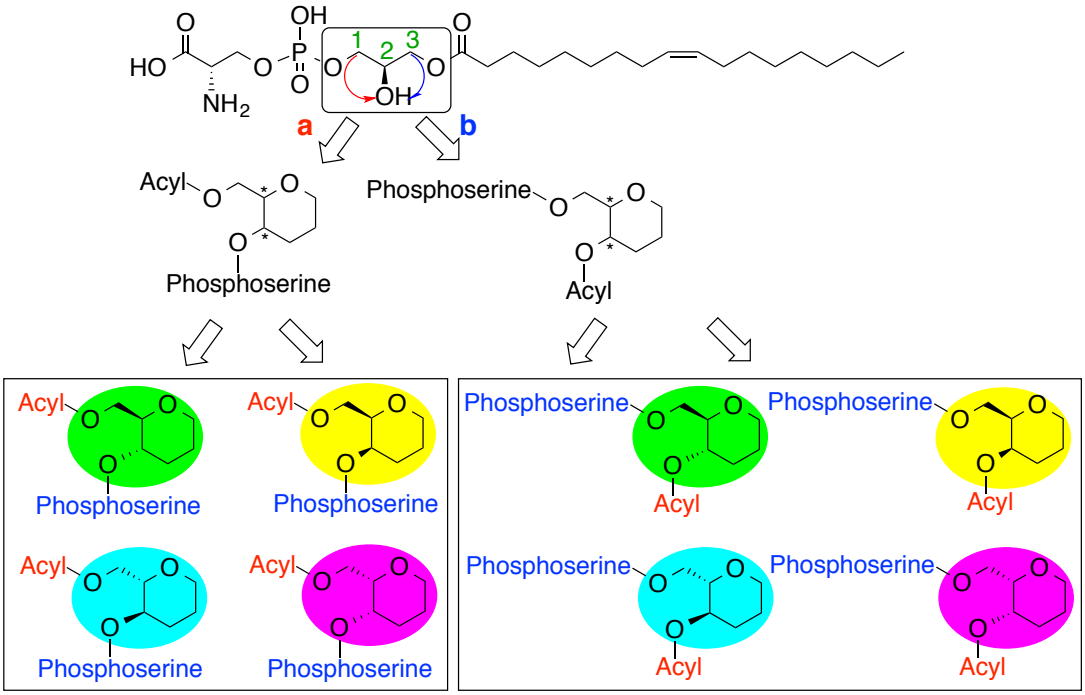


Figure 11. Molecular design and modification of glycerol framework- saturated cyclic analogue

In order to generate as much derivatives as we can obtain for this conformation study, we tried to introduce ring moiety containing noneuquivalent substitutents corresponing to the glycerol backbone, which make it possible to generate two different regioisomers, one of them containing acyl chain on the primary position, the other one containing acyl chain on the secondary position (Figure 11). Moreover, the stereochemistry at the ring junction also provides diversified cyclic analogues, trans/cis configuration and their enantiomers. Therefore, eight kinds of cyclic conformers were examined for this study (Figure 11).

Furthermore, the unsaturated intermediate, which has a double bond inside of the ring obtained during the synthetic procedure, is also utilized (Figure 12). Therefore, four kinds of saturated cyclic conformers and four unsaturated cyclic confomers are examined in this study.

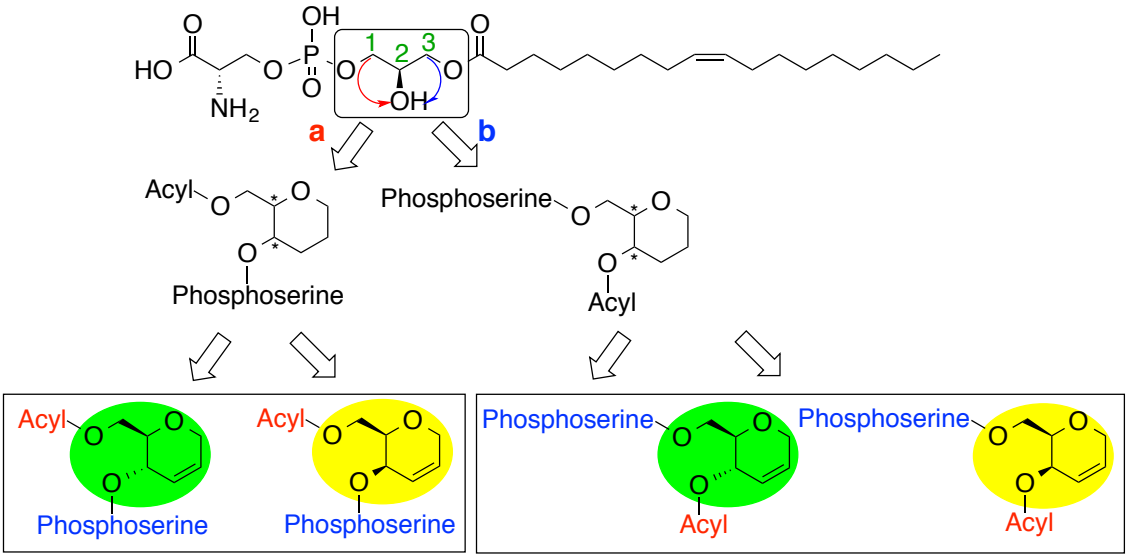


Figure 12. Molecular design and modification of glycerol framework

; Unsaturated cyclic analogue

With respect to the fatty acid, three kinds of fatty acid chains are used in this study (Figure 13). **A** is one of the components of lysoPS and composed of only hydrocarbon. **B** and **C** are its surrogates containing benzene ring connected by ether

linkage. They were chosen for this study because the lysoPS analogues containing **B** and **C** expressed potent activities against P2Y10 and GPR34 respectively and it is expected to afford selectively potent analogues toward each receptor. Additionally, flexibility and hydrophobicity change are expected by introducing fatty acid surrogates, **B** and **C**.

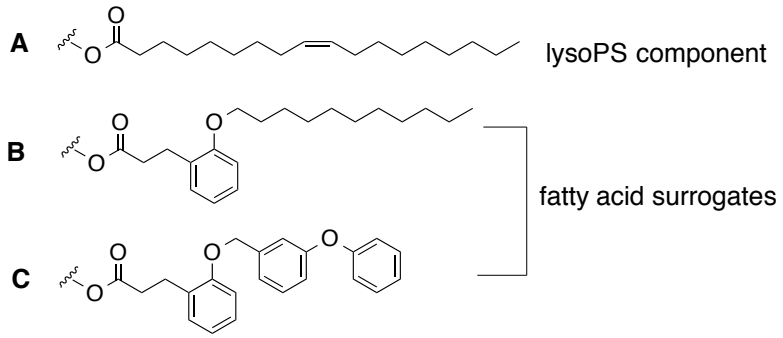


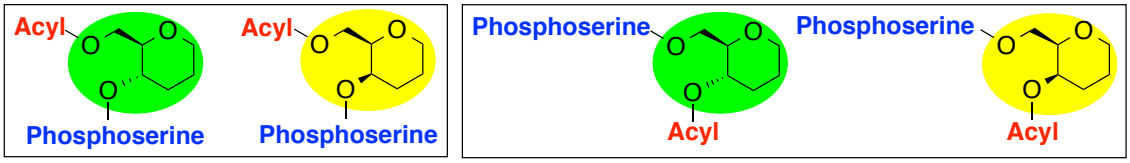
Figure 13. Fatty acid and its surrogates used in this study and their abbreviations

The names of cyclic lysoPS analogues are defined here. They will be described the configuration of trans or cis first, followed by substituent and its position, primary or secondary. That is, (trans / cis)-(1˚ / 2˚)-(Acyl / Phosphoserine)-(1˚ / 2˚)-(Acyl / Phosphoserine).

Results and Discussions

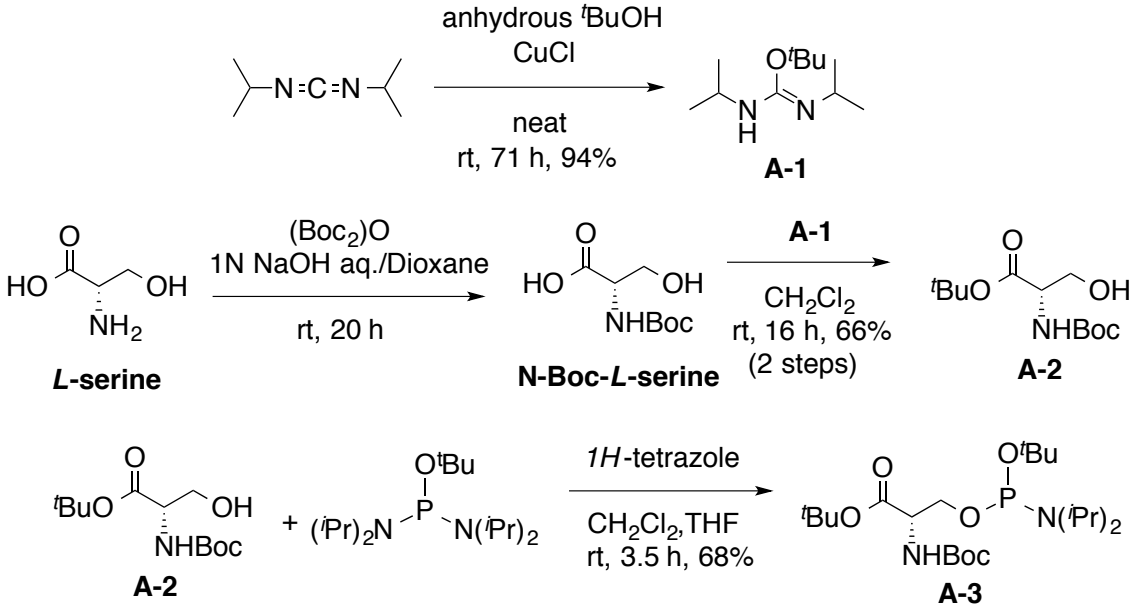
Chapter 3. The Effect of Constrained Glycerol Framework on Activity and Subtype-Selectivity and Elucidation of Active Conformation of lysoPS against GPR34

3-1. Synthesis of Saturated Cyclic LysoPS Analogues



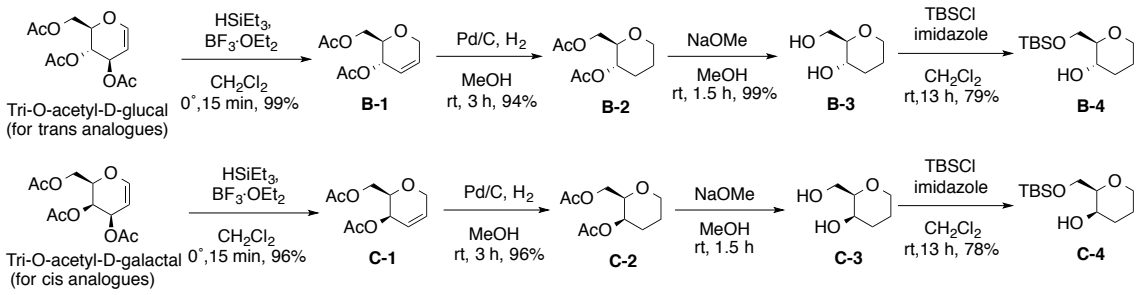
(from **Figure 11**)

Designed cyclic lysoPS analogues shown above are synthesized. First of all, the phosphoserine was protected by tertiary butyl and Boc group, which are deprotected at once under TFA condition on the last step of generation of lysoPS analogues. Formation of phosphodiester bond is carried out by phosphoramidite method to give **A-3** (Scheme 1).



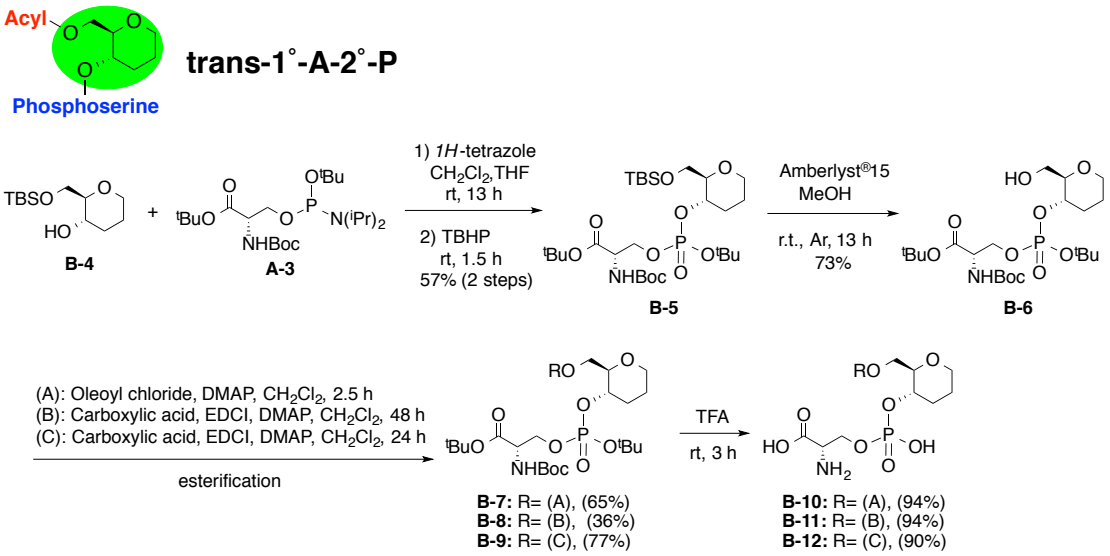
Scheme 1. Synthesis of phosphoserine unit

Continuously, modified glycerol backbone, 2-hydroxymethyl-3-hydroxytetrahydropyran moiety, was synthesized using the commercially available sugar compounds, tri-O-acetyl-D-glucal for trans, tri-O-acetyl-D-galactal for cis, which are readily determined their stereochemistry[26, 27]. The starting sugars are treated by ionic hydrogenation ($HSiEt_3$ and $BF_3{\cdot}OEt_2$), followed by hydrogenation over Pd/C, and then hydrolysis of two acetyl groups through the reaction using NaOMe/MeOH, established the structure of trans- and cis-2-hydroxymethyl-3-hydroxytetrahydropyrans, respectively (Scheme 2).

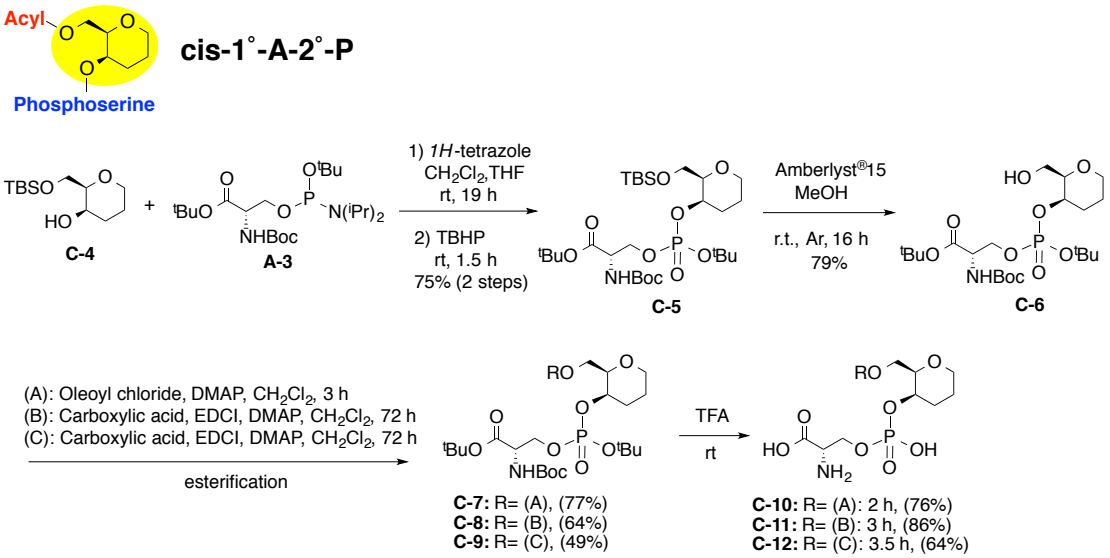


Scheme 2. Synthesis of modified cyclic glycerol backbone

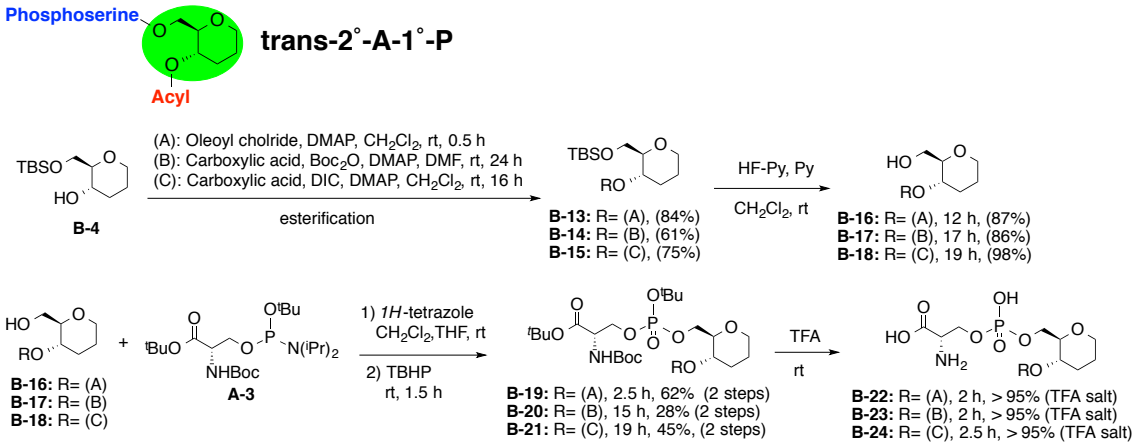
A primary alcohol was selectively protected with a TBS group, followed by formation of a phosphate diester linkage through the phosphoramidite method. Acid-catalyzed deprotection of the silyl ether was carried out to give a free primary alcohol, followed by acylation with a various fatty acid analogues, and complete deprotection in TFA furnished each lysoPS analogue, 1˚-acyl-2˚-phosphoserine (Scheme 3 for trans and Scheme 4 for cis). 2˚-acyl-1˚-phosphoserine compounds were also obtained by simple change of the reaction order of the esterification and the connection of the phosphoserine (**A-3**) (Scheme 5 for trans and Scheme 6 for cis).



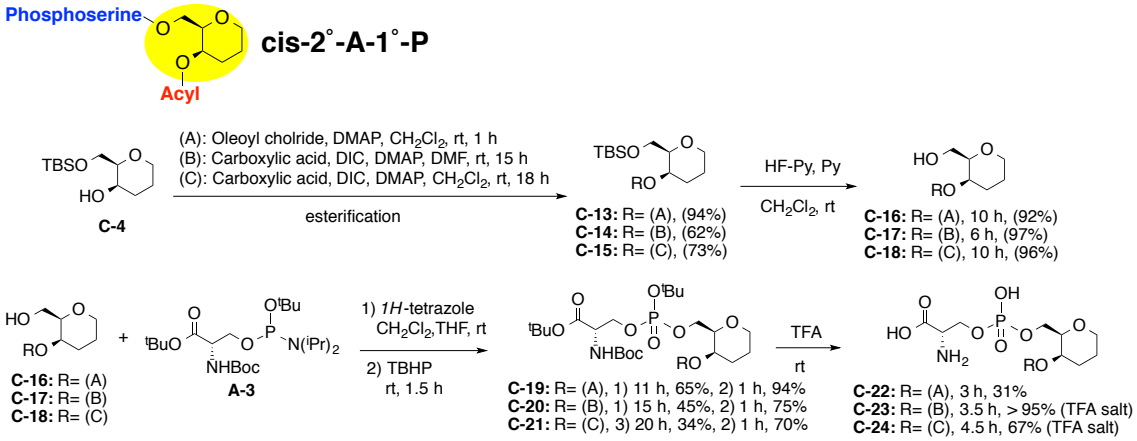
Scheme 3. Synthesis of trans-1˚-acyl-2˚-phosphoserine



Scheme 4. Synthesis of cis-1˚-acyl-2˚-phosphoserine



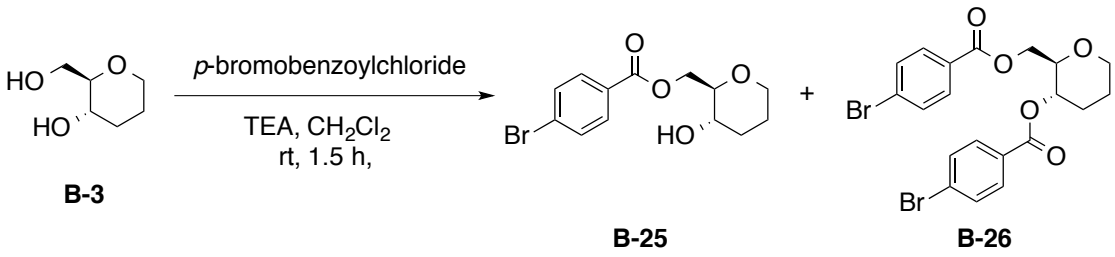
Scheme 5. Synthesis of trans-2˚-acyl-1˚-phosphoserine



Scheme 6. Synthesis of cis-2˚-acyl-1˚-phosphoserine

3-2. Conformational Analysis in Solution

In order to figure out the conformation of the obtained sugar derivatives, esterification of **B-3** was carried out using *p*-bromobenzoylchloride to make single crystal for X-ray diffraction analysis (Scheme 7). **B- 25** and **B-26** were obtained and recrystallized in ethanol as a solvent. Fortunately, we obtained the suitable crystal for trans configuration and got its X-ray crystal structure (Figure 14). As a result, it is disclosed that trans configuration takes diequatorial. In addition, it was confirmed that tetrahydropyrne ring and benzene ring are placed with perpendicular manner from each other (Figure 14).



Scheme 7. Synthesis of **B-25** and **B-26** for X-ray crystal structure analysis

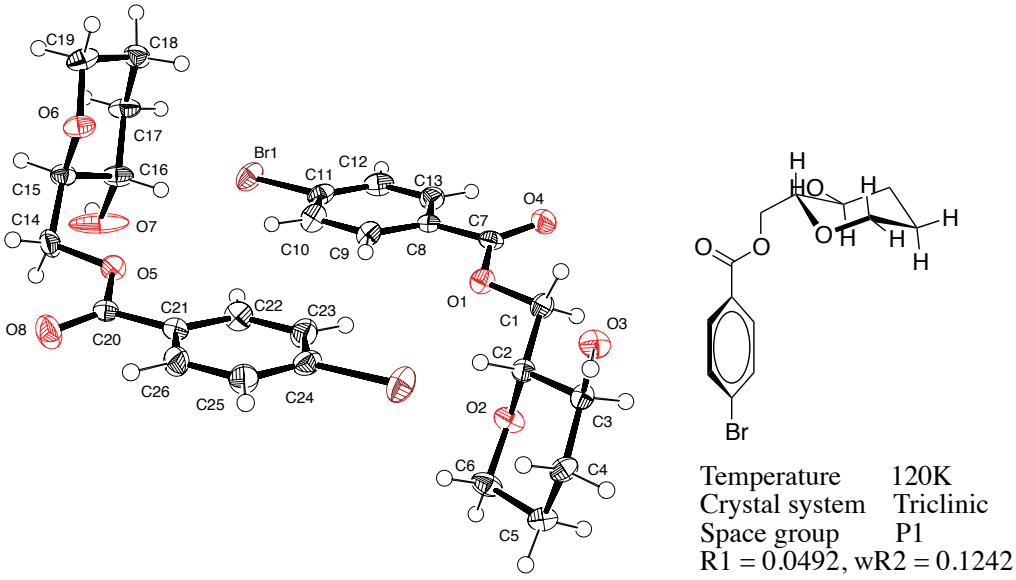


Figure 14. X-ray crystal structure of compound **B-25**

In the case of cis conformation, we could not obtain the crystal, but examined its structure in solution state ($CDCl_3$) using the compound, which has modified acyl chain (C). It is confirmed that the NOE between H1 and H3 should be observed when the compound has primary on equatorial and secondary on axial position. If the compound has primary on axial and secondary on equatorial position, the NOE between H2 and H4 / H3 and H6 should be detected. The former NOE was observed and it was determined that primary position is equatorial and secondary position is axial (Figure 15).

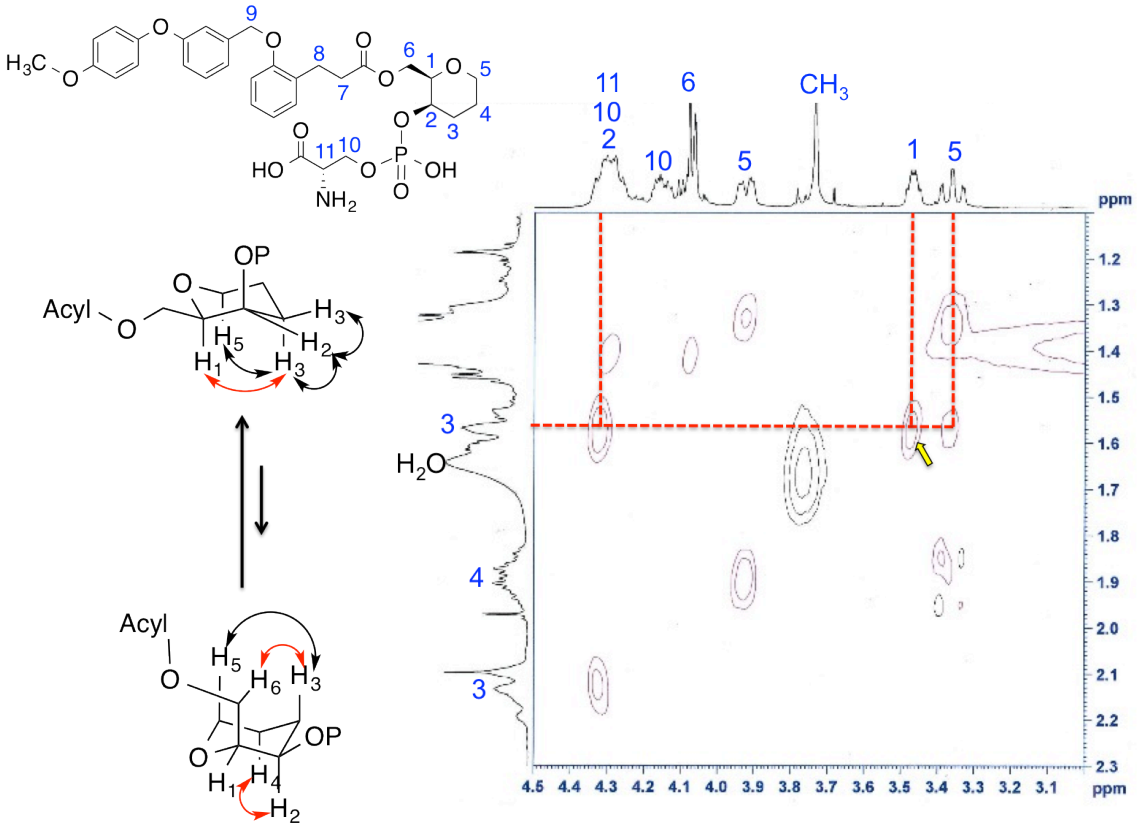


Figure 15. NOE of modified compound **C-12** (400MHz, $CDCl_3$)

3-3. Shedding Assay Results

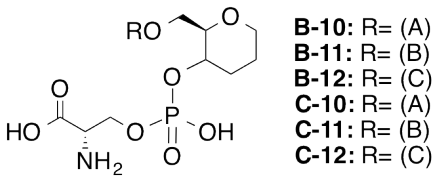
3-3-1. Effect of Regioisomerisms

The all synthesized analogues were evaluated their agonistic activities by TGF α shedding assay and the results are shown on Table 1.

As we expected, the agonistic activity against GPR174 was disappeared in the case of cyclic lysoPS analogues. trans-1˚-(A)-2˚-P (**B-10**) (P is abbreviation of phosphoserine.) is around twice more potent than lysoPS against GPR34 and trans-1˚-(C)-2˚-P (**B-12**) has similar potency to lysoPS itself toward GPR34 (Table 1, Figure 16). In addition, they show weak or almost no activity to P2Y10. From these results, both are increased their selectivity by fixation of hydroxyl group on sn-2 position of glycerol on lysoPS. In the case of cis conformer, cis-1˚-(A)-2˚-P (**C-10**) and cis-1˚-(C)-2˚-P (**C-12**) have potent agonistic activities against GPR34 with high selectivity, especially, **C-12** is strong agonist against GPR34, which is four times more potent than lysoPS itself. This result is consistent with what we recognized in the case of linear lysoPS analogue (Table 1, Figure 16), which contains acyl chain (C) and expresses the strongest agonistic activity against GPR34.

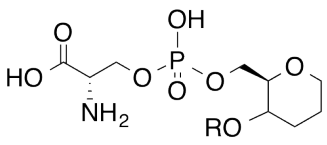
Moreover, **B-11** and **C-11** have relatively strong agonistic activity against P2Y10 together with weak or almost no activity toward GPR34. Considering these shedding results, it is expected that P2Y10 activity is derived from the sub-type selectivity of acyl chain (B), which is effective for P2Y10 realized from the previous study. Additionally, these results imply that cis configuration is better for GPR34 activation whereas trans is effective for P2Y10 activation from comparison between **B-11** and **C-11**.

Table 1. Shedding assay results of trans and cis 1˚-acyl-2˚-phosphoserine (1˚-acyl-2˚-P) and 2˚-acyl-1˚-phosphoserine (2˚-acyl-1˚-P)



B-10: R= (A)
B-11: R= (B)
B-12: R= (C)
C-10: R= (A)
C-11: R= (B)
C-12: R= (C)

1˚-acyl-2˚-P



B-22: R= (A)
B-23: R= (B)
B-24: R= (C)
C-22: R= (A)
C-23: R= (B)
C-24: R= (C)

2˚-acyl-1˚-P

trans					*trans*				
		GPR34	P2Y10	GPR174			GPR34	P2Y10	GPR174
(A)	B-10	130 nM	807 nM	inactive	(A)	B-22	1600 nM	inactive	inactive
(B)	B-11	>10 μM	106 nM	inactive	(B)	B-23	inactive	inactive	inactive
(C)	B-12	228 nM	6.5 μM	inactive	(C)	B-24	1200 nM	1800 nM	inactive
cis					*cis*				
		GPR34	P2Y10	GPR174			GPR34	P2Y10	GPR174
(A)	C-10	170 nM	inactive	inactive	(A)	C-22	1600 nM	inactive	inactive
(B)	C-11	200 nM	196 nM	inactive	(B)	C-23	inactive	inactive	inactive
(C)	C-12	54 nM	inactive	inactive	(C)	C-24	98 nM	inactive	inactive

Cf.

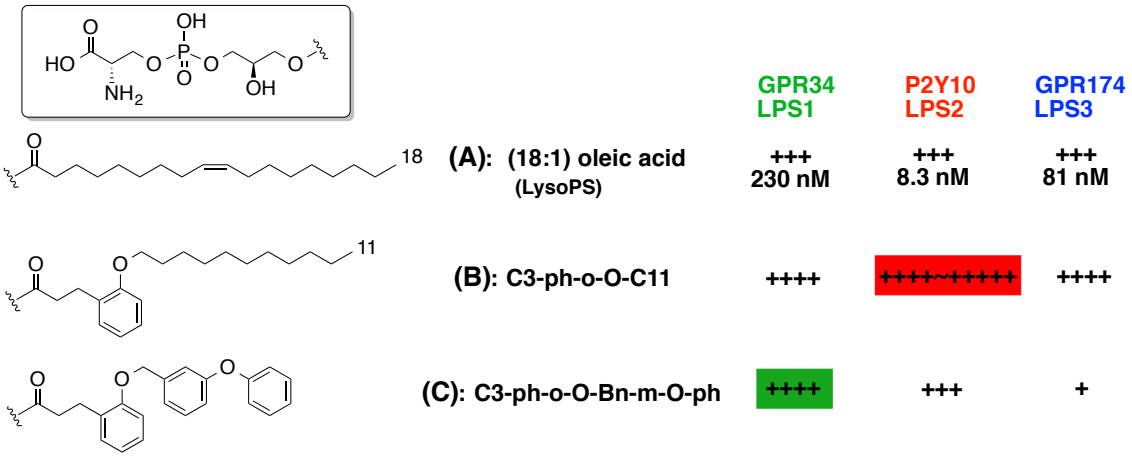


Figure 16. Shedding assay results of representative lysoPS analogues

3-3-2. Effects of Shape of Fatty Acids

Continuously, the cyclic analogues of 2˚-acyl-1˚-P series are almost no activity against all receptor except **C-24**, which is around twice more potent than lysoPS itself toward GPR34. To explain this exceptional result, we first investigated acyl chain (C) on cis configuration by transformation of (C) to its isomers (Figure. 17, 18) for **C-12** and **C-24** and the results shown in Table 2, Figure 19 and 20.

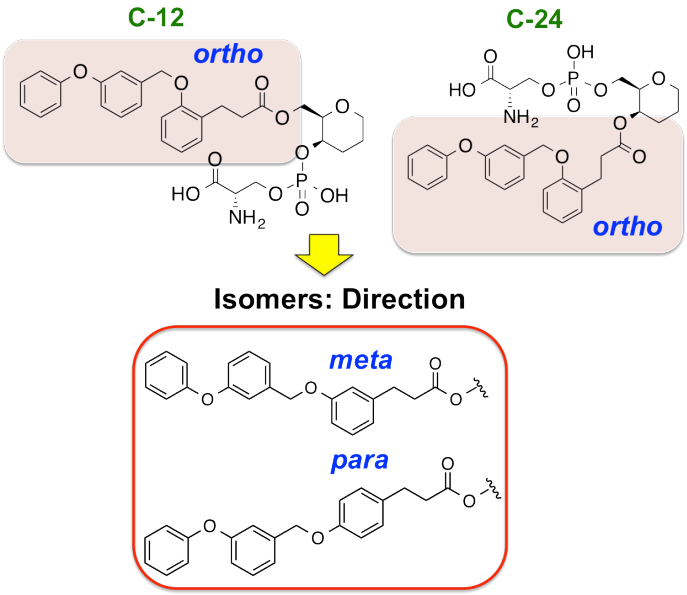


Figure 17. Examination of meta and para-isomers of acyl chain (C)

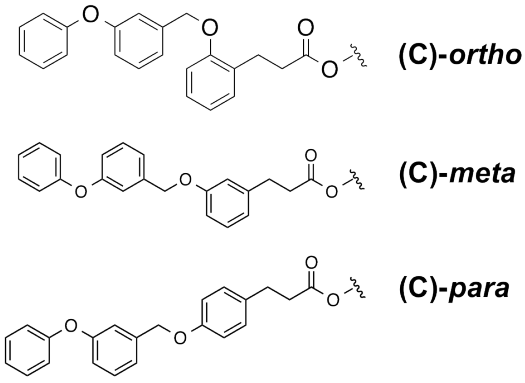
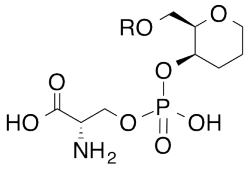
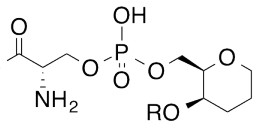


Figure 18. Modification of the acyl chain (C); *meta* and *para* isomers

Table 2. Shedding results of the analogues containing acyl chain (C) isomers



C-12: R= (C)-*ortho*
C-25: R= (C)-*meta*
C-26: R= (C)-*para*



C-24: R= (C)-*ortho*
C-27: R= (C)-*meta*
C-28: R= (C)-*para*

1˚-acyl-2˚-P 2˚-acyl-1˚-P

	cis				*cis*		
	GPR34	P2Y10	GPR174		GPR34	P2Y10	GPR174
C-12	54 nM	inactive	inactive	**C-24**	98 nM	inactive	inactive
C-25	inactive	inactive	inactive	**C-27**	inactive	inactive	inactive
C-26	inactive	inactive	inactive	**C-28**	inactive	inactive	inactive

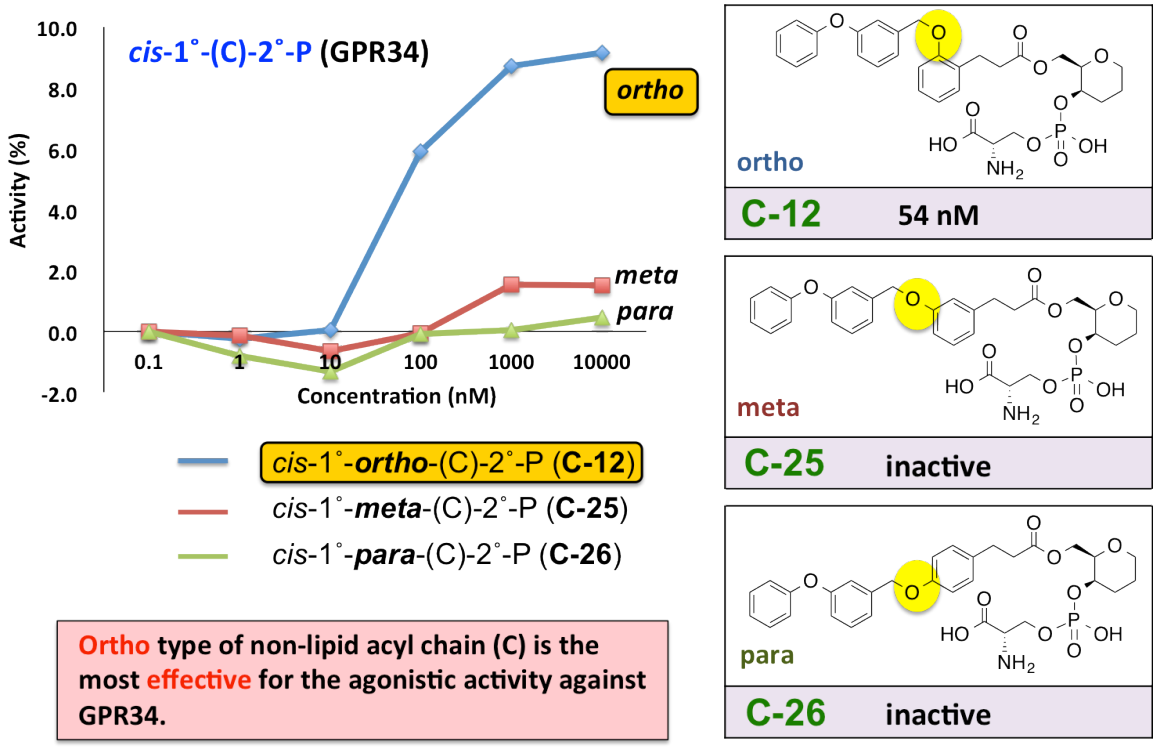


Figure 19. Shedding assay results of meta and para-isomers of **C-12** against **GPR34**

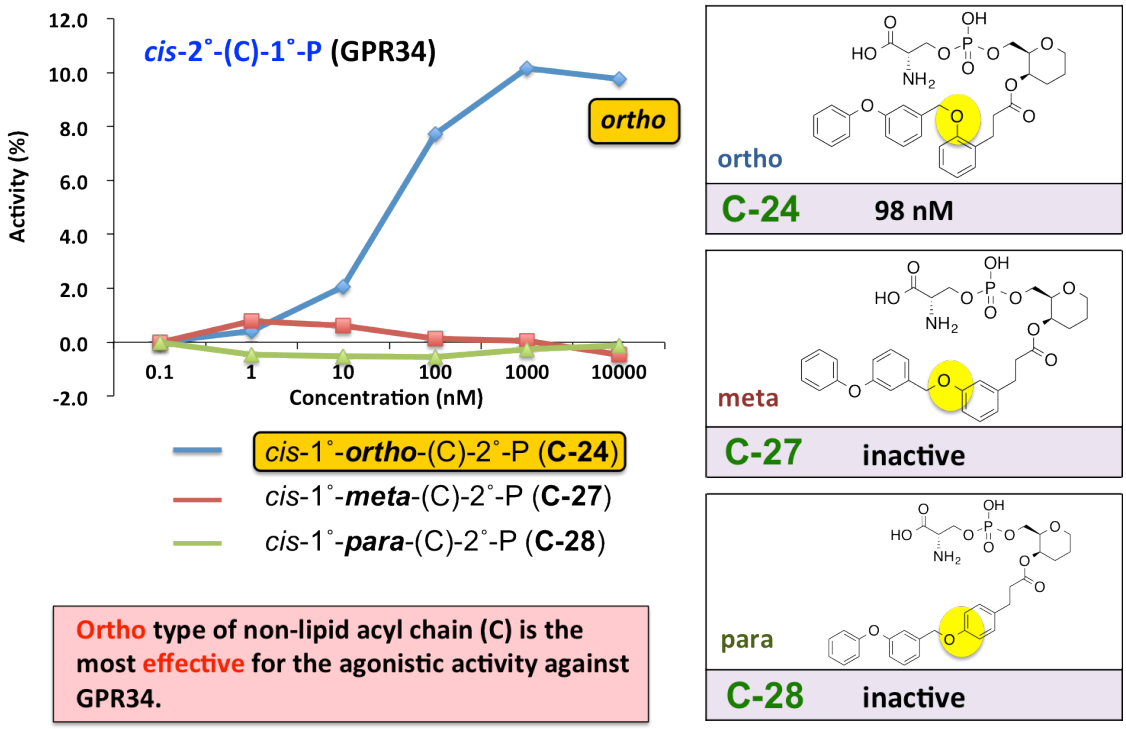


Figure 20. Shedding assay results of meta and para-isomers of **C-24** against **GPR34**

The results suggested that *ortho* type of acyl chain (C) of **C-12** is the most essential for GPR34 activation comparing to the *meta* and *para* isomers, which make the agonistic activity eliminated (Figure 19). This result is also applicable to **C-24** including (C) on the secondary position and its activity is similar to that of **C-12** (Figure 20). Considering this result, it is required for the second benzene ring of acyl chain (C) to connect to the first benzene ring on the ortho position, which might be important to induce favorable conformation for GPR34 activation.

3-3-3. Effects of Aromatic Substituents in the Fatty Acids

Furthermore, we tried to investigate its generality by introducing substituents, chlorine and methyl, on the terminal benzene ring of acyl chain (C) (Figure 21, 22) and the results are shown in Table 3, Figure 23 and 24.

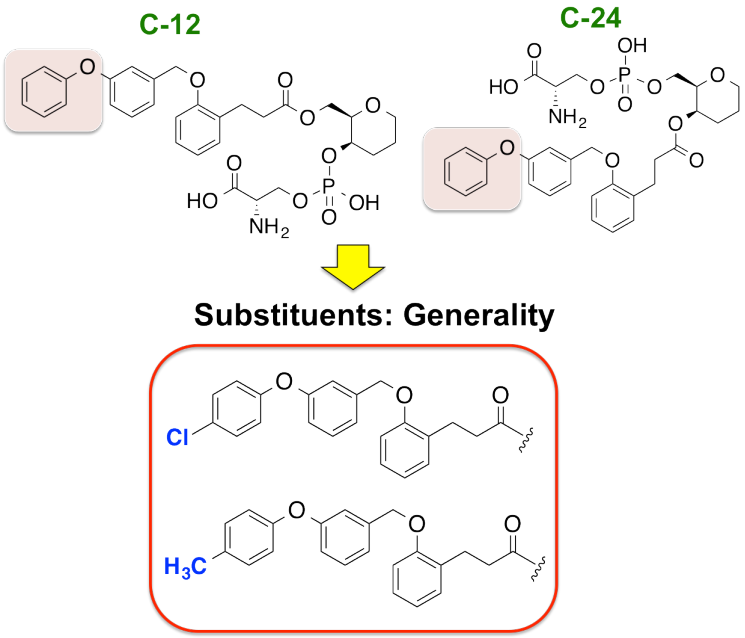


Figure 21. Examination of generality of **C-12** and **C-24** by introducing substituent to the terminal benzene ring of acyl chain (C)

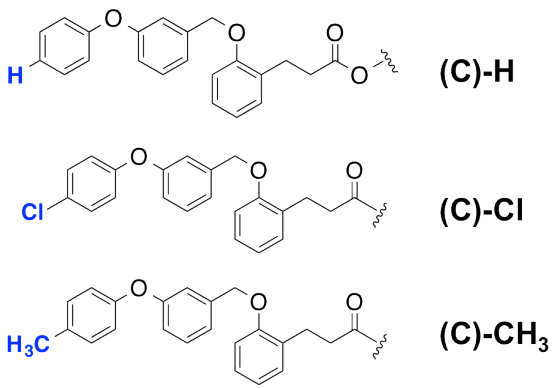
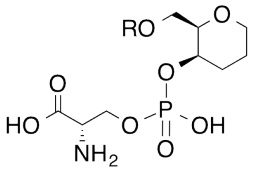
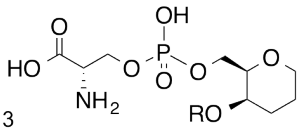


Figure 22. Modificationof acyl chain (C); introduction of substituent to the terminal benzene ring of (C)

Table 3. Shedding assay results of the analogues containing acyl (C)-*para* substituents



C-12: R= (C)-H
C-29: R= (C)-Cl
C-30: R= (C)-CH_3



C-24: R= (C)-H
C-31: R= (C)-Cl
C-32: R= (C)-CH_3

1˚-acyl-2˚-P　　2˚-acyl-1˚-P

cis				*cis*			
	GPR34	P2Y10	GPR174		GPR34	P2Y10	GPR174
C-12	54 nM	inactive	inactive	**C-24**	98 nM	inactive	inactive
C-29	35 nM	inactive	inactive	**C-31**	70 nM	inactive	inactive
C-30	28 nM	inactive	inactive	**C-32**	42 nM	inactive	inactive

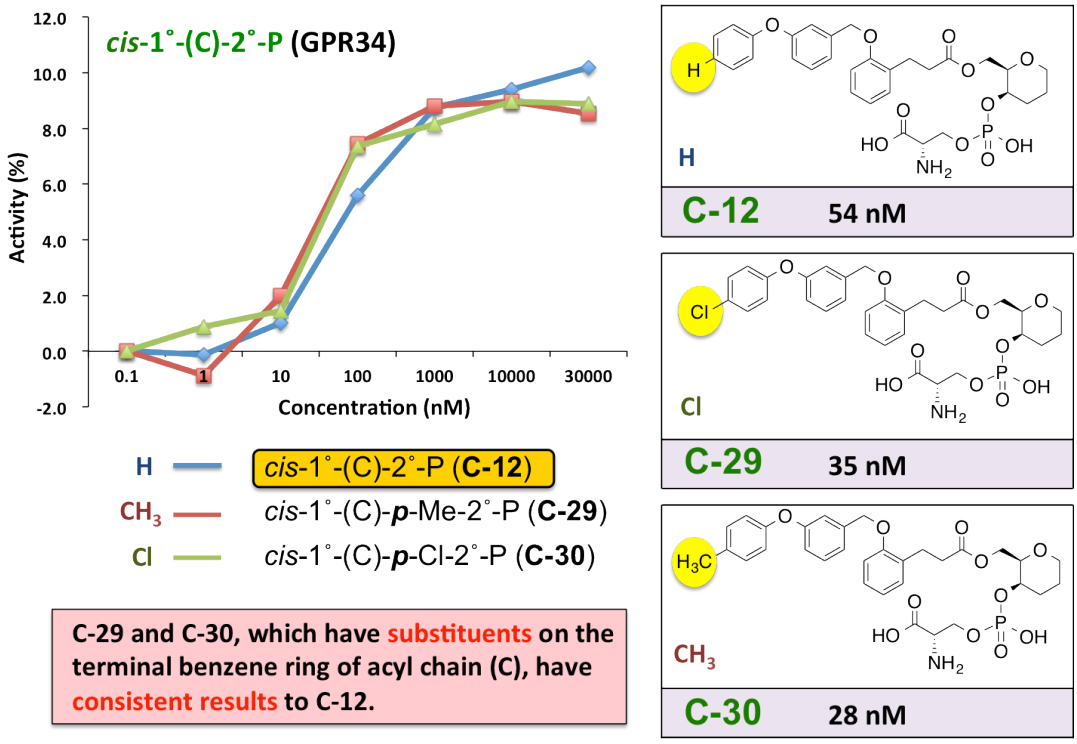


Figure 23. Assay results of **C-12** for examination of its generality against **GPR34**

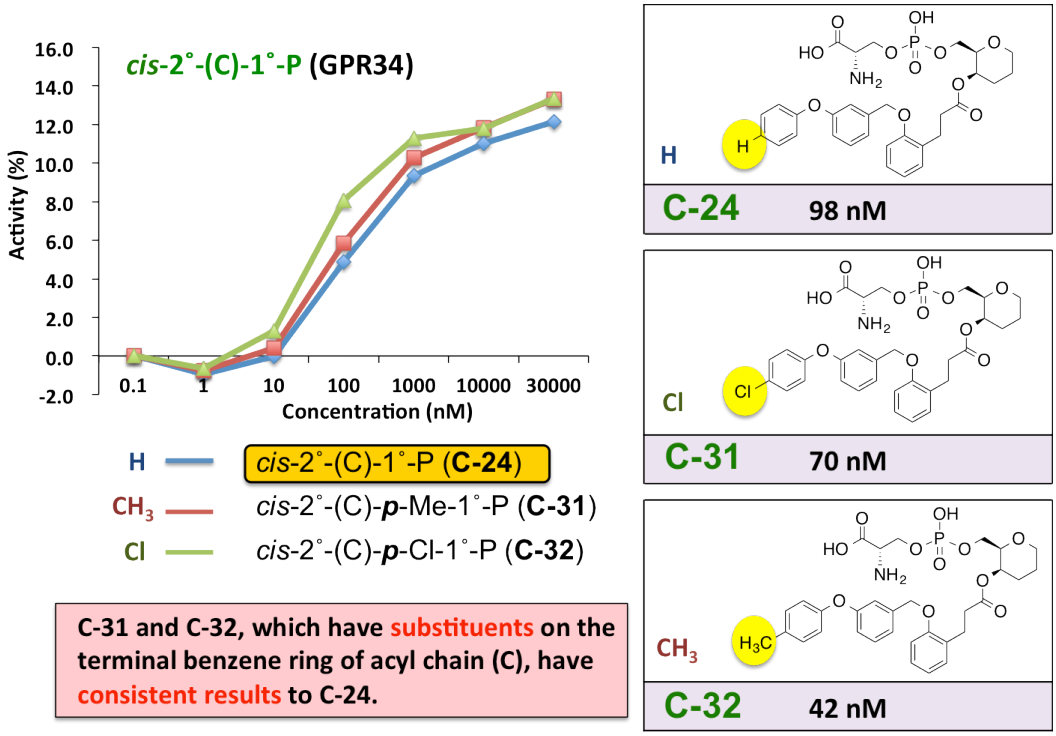


Figure 24. Assay results of **C-24** for examination of its generality against **GPR34**

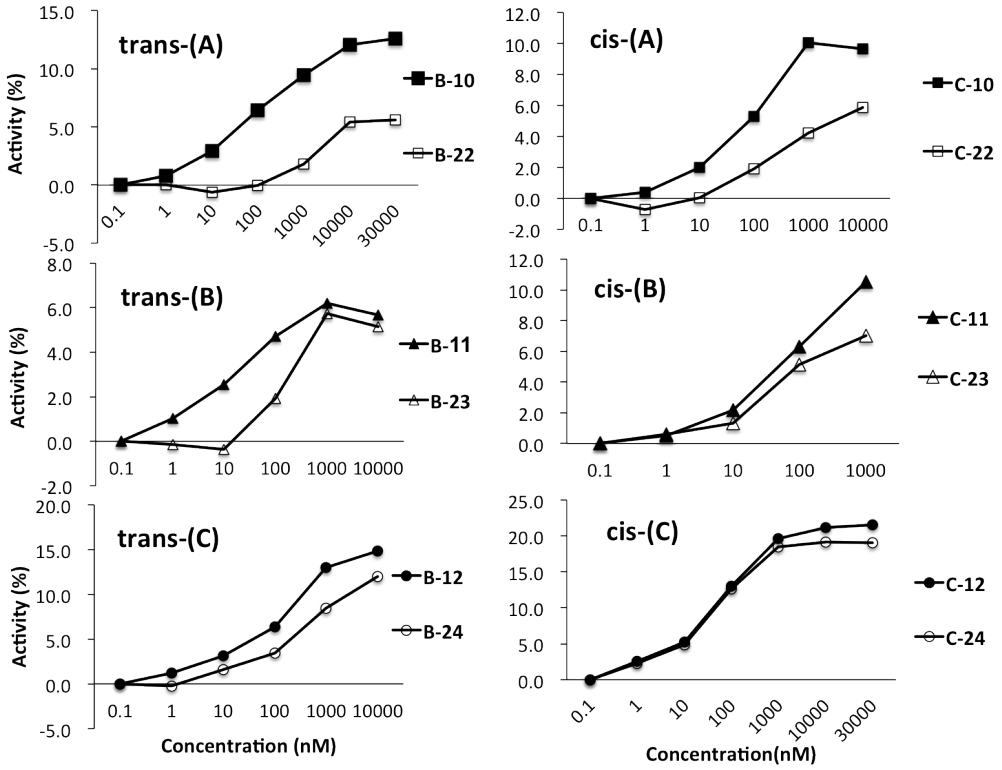


Figure 25. Concentration-response curve of trans and cis-1˚-acyl-2˚-phosphoserine and 2˚-acyl-1˚-phosphoserine

The results suggested that not only primary acyl compounds, **C-29** and **C-30** express strong potency, even more potent, but also secondary acyl compounds, **C-31** and **C-32** have high potency against GPR34 (Figure 23, 24). These results indicated that the exceptional result of **C-24** agonistic activity toward GPR34 is consistent with the **C-31** and **C-32**. The result provided that **C-24** is possible to take similar conformation to that of **C-12** for GPR34 activation. We thought that this is plausible in the case of the analogues containing acyl chain (C), which has reduced flexibility than acyl chain (A) and (B) and suppress dynamic conformation changes.

Furthermore, the shedding assay results so far suggested that primary acyl chain compounds are more effective for receptor activation in general (Figure 25). Taking the conformation into account, secondary carbon at the ring junction is a component of tetrahydropyran ring and limited to rotate, which means more constrained than freely rotatable primary carbon. This indicated that multiply changeable acyl chain is effective for receptor activation.

3-4. Calculation Study

3-4-1. Effect of Regioisomerism of Tetrahydropyrane Derivatives by Examination of Torsion Angle of the Ring

The structures of our target GPCRs are still unknown. Therefore, we tried to consider the active confomers of ligand from point of view of restricted ligand structures and hypothesized that lysoPS can take similar conformations to lysoPS analogues, which are potent and selective against each single receptor. Therefore, we carried out calculation study to figure out structural features of cyclic lysoPS analogues and lysoPS itself in order to compare them to confirm whether they have similar conformations or not.

For this study, MD simulation was carried out to define accessible conformations of cyclic lysoPS analogues. Continuously, to find conformational characteristics of cyclic analogues, we tried to calculate the dihedral angle, ∠O-C-C-C, which is represented with red color in Figure 26.

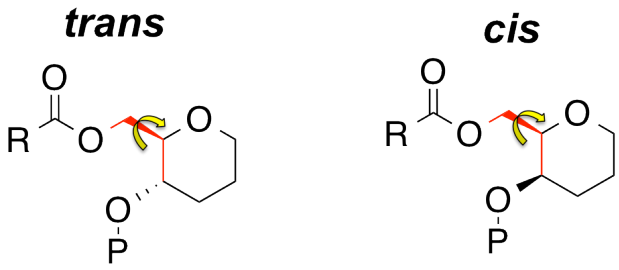


Figure 26. Calculated dihedral angle at the ring junction

It is predicted that this examination will give some information to classify general conformation because overall structure would be changed considerably depending on the direction of the primary substituent. In other words, primary carbon at the ring junction is rotatable and makes it possible to take various conformations. On

the other hand, secondary position is restricted by ring moiety and it would not affect critical conformation changes. The conformational changes of the tetrahydropyran ring framework and how to divide the calculated dihedral angles are shown in Figure 27.

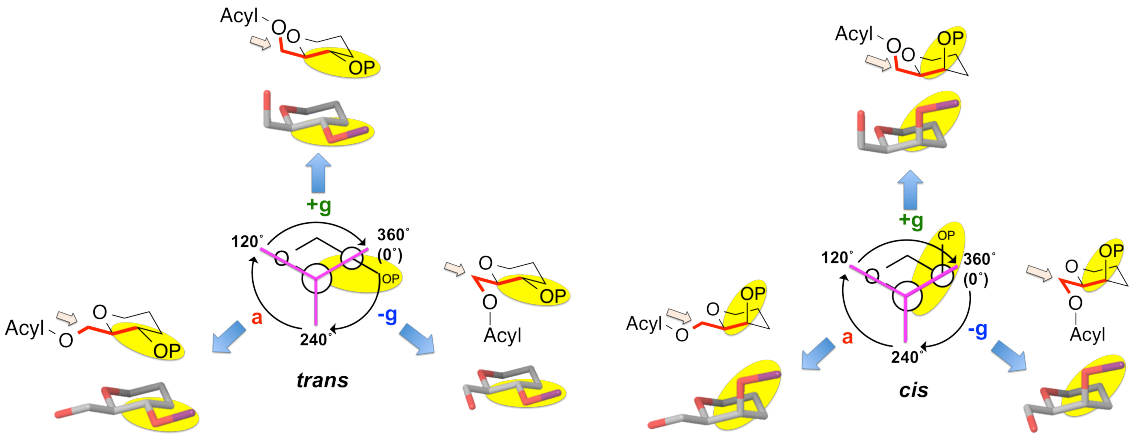


Figure 27. Conformational change of ring moiety and definition of dihedral angles

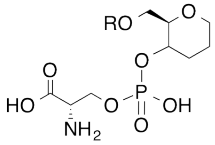
The range of angle is divided to three sections from 0˚ to 360˚ by every 120˚ and every part of ranges are named **+g** (0˚ to 120˚), **a** (120˚ to 240˚) and **–g** (240˚ to 360˚). If the primary substituent is located anti position to the phosphoserine part, it is named **a** (120˚ to 240˚). If the primary substituent turns clockwise, it is named **+g** (0˚ to 120˚). Lastly, the name of **–g** (240˚ to 360˚) is for the primary substituent turned counter clockwise to the phosphoserine unit.

In the case of trans (**B-10**, **B-11** and **B-12**), the substituent on the secondary position is located on equatorial whereas cis (**C-10**, **C-11** and **C-12**) have the secondary substituent on axial position, which are marked with yellow color (Figure 27). The calculation results of dihedral angles are represented as percentage of distribution (Table 4).

Table 4. Distributions of dihedral angle and their percentage

	trans			*cis*		
	B-10	B-11	B-12	C-10	C-11	C-12
GPR34 (EC_{50})	130 nM	>10 μM	228 nM	170 nM	200 nM	54 nM
-g	1.7 %	0.6 %	0.6 %	1.5 %	0.9 %	3.9 %
a	82.1 %	92.5 %	91.5 %	98.0 %	98.8 %	95.9 %
+g	16.3 %	6.9 %	7.9 %	0.4 %	0.3 %	0.3 %

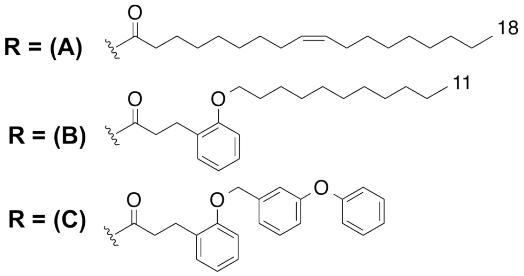
Cf. from **Table 1**



B-10: R= (A)
B-11: R= (B)
B-12: R= (C)
C-10: R= (A)
C-11: R= (B)
C-12: R= (C)

1˚-acyl-2˚-P

trans				
		GPR34	P2Y10	GPR174
(A)	B-10	130 nM	807 nM	inactive
(B)	B-11	>10 μM	106 nM	inactive
(C)	B-12	228 nM	6.5 μM	inactive
cis				
		GPR34	P2Y10	GPR174
(A)	C-10	170 nM	inactive	inactive
(B)	C-11	200 nM	196 nM	inactive
(C)	C-12	54 nM	inactive	inactive



As the table shown above, both trans and cis have the highest percentage of **a** where trans has **+g** and **–g** as last in order, cis has **–g** and **+g** on the contrary to trans. As a result, it seems that **+g** and **–g** are the important factors to explain difference between trans and cis, which is formulated as a hypothesis showing relationship of dihedral angle and activity.

In other words, the agonistic activities of **B-10** against GPR34 is the most potent among trans analogues, next is **B-12** and **B-11** is the last. Continuously, as shown in Table 4, percentage distribution of **B-11** on row **a**, marked by red square on trans configuration, is the highest, next is **B-12** and **B-10** is the last in order. The order is opposite to **a** for **+g** case.

Considering the order together with that of activity, **a** is opposite to activity order whereas **+g** has the same order as that of activity. Considering from conformation point of view, highest percentage of **a** is reasonable because anti position between two substituents make the steric hindrance decreased and lead to more stable conformations. However, on the basis of the assumption that active conformation is not always the most stable structure because a ligand should overcome the energy barrier to bind to a receptor. Therefore, the conformations, which belong to the less distribution of **a**, are probably more responsible for active conformation. Moreover, **+g**, of which the order is the same as that of activity, is presumably important factor to affect active conformation. As shown in Figure 27, taking **+g** dihedral angle means that primary substituent, hydrophobic acyl chain, is located closer to the phosphoserine, polar head group than that on the anti position. This may lead to the result that overall structure can take folded structure, or the possibility of taking folded structure is increased at least. Thinking about this dihedral angle, low distribution of **a** and high distribution of **+g**, together with activity results, folded structure is probably accessible conformation toward GPR34.

In the case of cis, it also has the same tendency as that of trans configuration. It seems that **–g** instead of **+g** is involved in having approachable active conformation for cis. This is because cis configuration has the substituent on secondary position at the ring junction, with axial position and percentage of **–g** distribution is virtually the highest for the analogue, **C-12**, which is the most potent among cyclic analogues and the least distribution of **a**.

3-4-2. Examination of General Structures of Cyclic LysoPS Analogues and LysoPS by Measuring Distances of the Molecules

On the basis of the calculation results of the dihedral angle, folded structure of the cyclic lysoPS analogues are responsible for receptor activation. Therefore, we tried to understand the general structure of lysoPS analogues by measuring distances. The results are shown in Figures 28, 29 and 30.

The graphs show distributions of calculated distances, from several carbons of acyl chain to phosphine atom of the polar head group. To give an example of definition of calculated distance, the distance from carbon 4 on acyl chain to phosphine atom is described as C4P.

To say the results first, compound cis-1˚-(C)-2˚-P (**C-12**), which is potent and selective against GPR34, has higher distributions at shorter distance ranges through overall distances, even for the terminal carbon of the acyl chain (Figure 30). This means that compound cis-1˚-(C)-2˚-P (**C-12**) is folded and acyl chain and phosphoserine part would be located close from each other. From the Figure 28 and 29, the distributions of compounds, cis-1˚-(A)-2˚-P (**C-10**) and cis-1˚-(B)-2˚-P (**C-11**), are different from that of cis-1˚-(C)-2˚-P (**C-12**). When calculated distances of acyl chain are getting longer, distributions at longer distance range is getting increased whereas compound cis-1˚-(C)-2˚-P has higher distributions at shorter distance ranges. This result implies that it is possible for compound 1˚-(C)-2˚-P (**C-12**) to originally take much more folded structure than others.

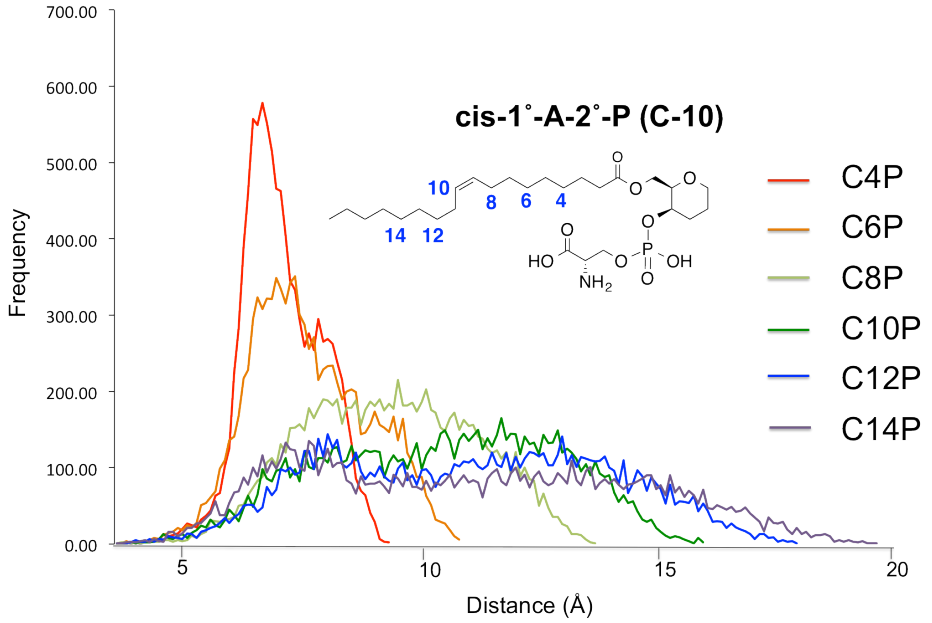


Figure 28. Distributions of distances between carbon atom on acyl chain to phosphine atom of polar head group; cis-1˚-A-2˚-P (**C-10**)

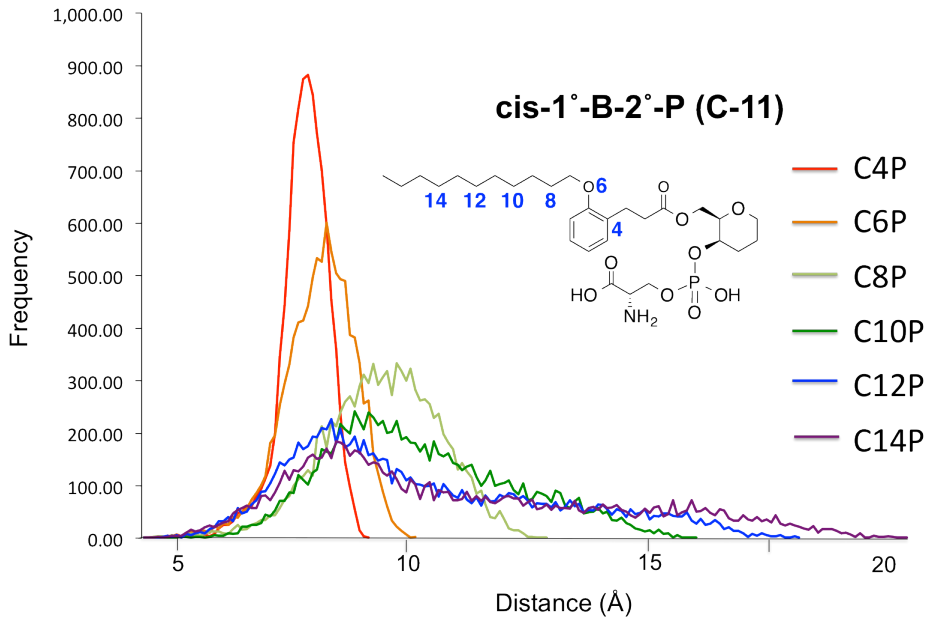


Figure 29. Distributions of distances between carbon atom on acyl chain to phosphine atom of polar head group; cis-1˚-B-2˚-P (**C-11**)

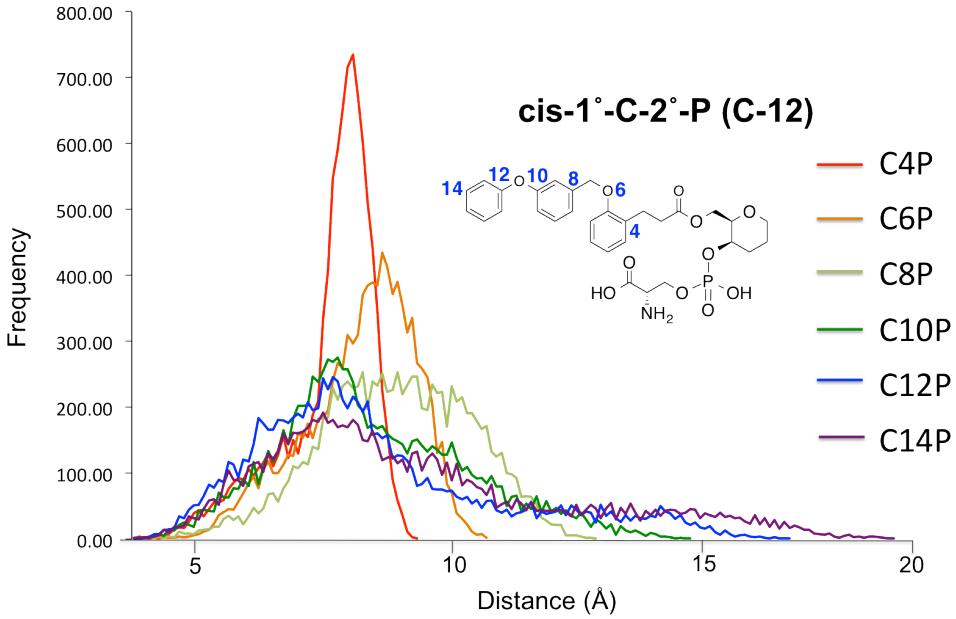


Figure 30. Distributions of distances between carbon atom on acyl chain to phosphine atom of polar head group; cis-1˚-C-2˚-P (**C-12**)

3-4-3. Comparison of 1˚-acyl-2˚-P vs. 2˚-acyl-1˚-P Analogues

In order to explain the present result that primary acyl chain analogues are more effective for GPR34 than secondary acyl analogues, we tried to compare between cis-1˚-(A)-2˚-P (**C-10**) and cis-2˚-(A)-1˚-P (**C-22**) (Figure 31). As shown in Figure 31, polar head group, phosphoserine is well superimposed and primary acyl chain is directed toward phosphoserine side (yellow arrow on **C-10**), which is possible to take folded structure. Moreover, the acyl chain on primary position is located closer to the phosphoserine than **C-22**. Even though secondary acyl chain of compound **C-22** is also rotated to direct to phosphoserine side, the conformation is probably not so favorable for GPR34 activation.

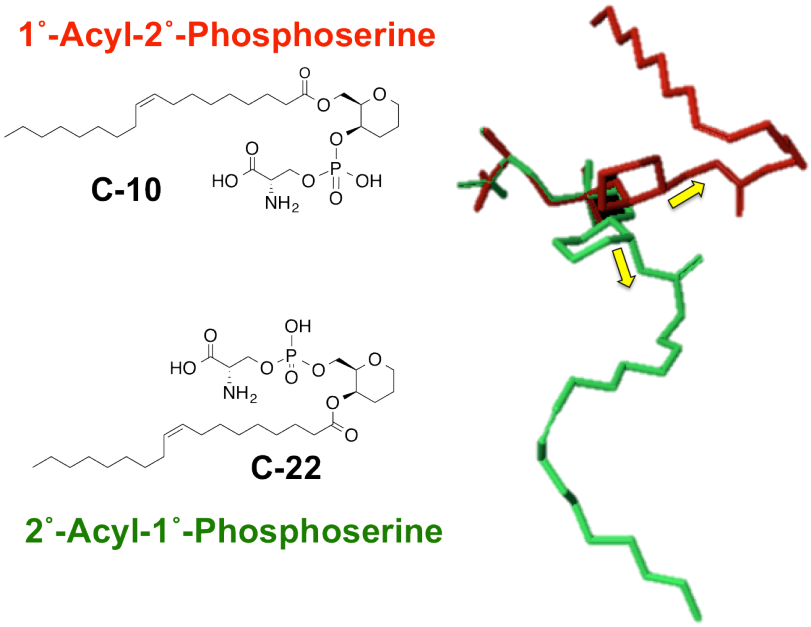


Figure 31. Superimpose image of 1˚-(A)-2˚-P and 2˚-(A)-1˚-P

However, in the case of **C-24**, which has acyl chain (C) on the secondary position, is exceptional. This could be explained by its rigidity. That is, acyl chain (C) is less flexible than acyl chain (A) and (B) and its possible conformations are confined to specific region in space. Thus, this special result is conceivable in the case of acyl chain (C).

3-4-4. Proposal of LysoPS Conformation Active for GPR34

To figure out structural features of lysoPS (1-oleoy lysophosphatidylserine), MD simulation was carried out to generate various lysoPS conformations probably including active conformation. After MD simulation, the obtained various conformations are clustered to three groups on the basis of root mean-square deviation (RMSD) value with its structural similarity (Figure 32). The cluster 1 contains folded structures of lysoPS, cluster 2 includes bending structures of lysoPS and relatively linear structures of lysoPS belong to cluster 3.

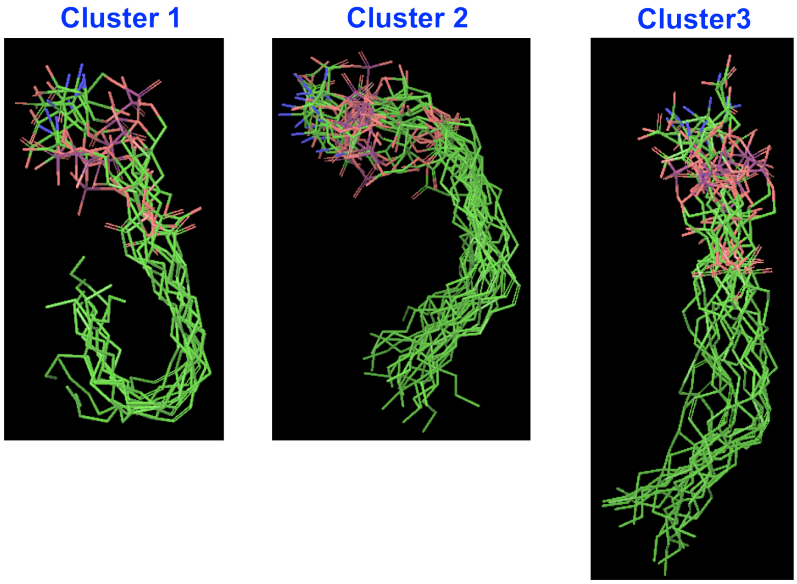


Figure 32. Clustering of lysoPS conformations (Total number of structure: 106. Number of cluster 1: 34. Cluster 2: 44. Cluster 3: 22.)

To begin with, the obtained lysoPS conformations are compared to the cyclic analogue **C-12**, which is potent and selective against GPR34. The results suggested that the structure of lysoPS, which belong to cluster 1, is similar to folded structure of **C-12** and it is revealed that **C-12** is well superimposed on the folded lysoPS conformation (RMSD = 5.7 ~ 7.0 Å for cluster 1 / 6.4 ~ 7.7 Å for cluster 2 / 7.3 ~ 8.4 Å for cluster 3)(Figure 33). Moreover, it shows that acyl chain is located close to the phosphoserine

unit, which is one of the causes of taking folded structure. The superimposed image is shown in Figure 34.

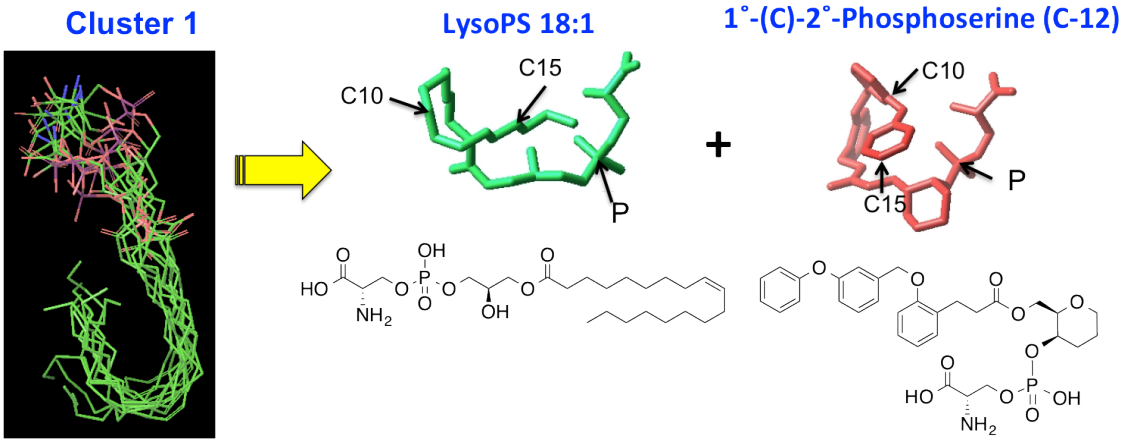


Figure 33. Comparison of lysoPS to the cyclic analogue **C-12**

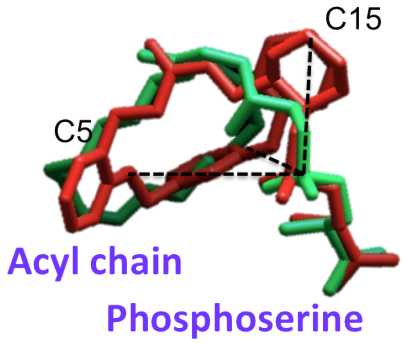


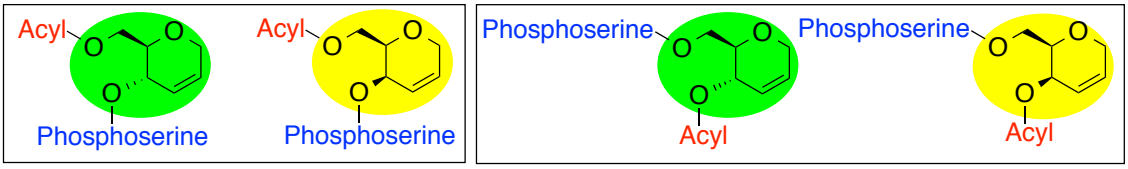
Figure 34. Superimposed image of **C-12** and lysoPS, which belong to cluster 1

Chapter 4.

Elucidation of Active Conformation of Lysophosphatidylserine against P2Y10

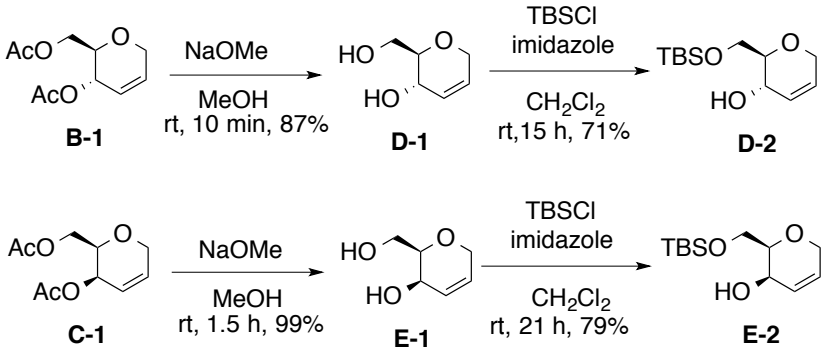
4-1. Effect of Unsaturation of Tetrahydropyran Framework on Receptor Activation

4-4-1. Synthesis of Unsaturated Cyclic LysoPS Analogues

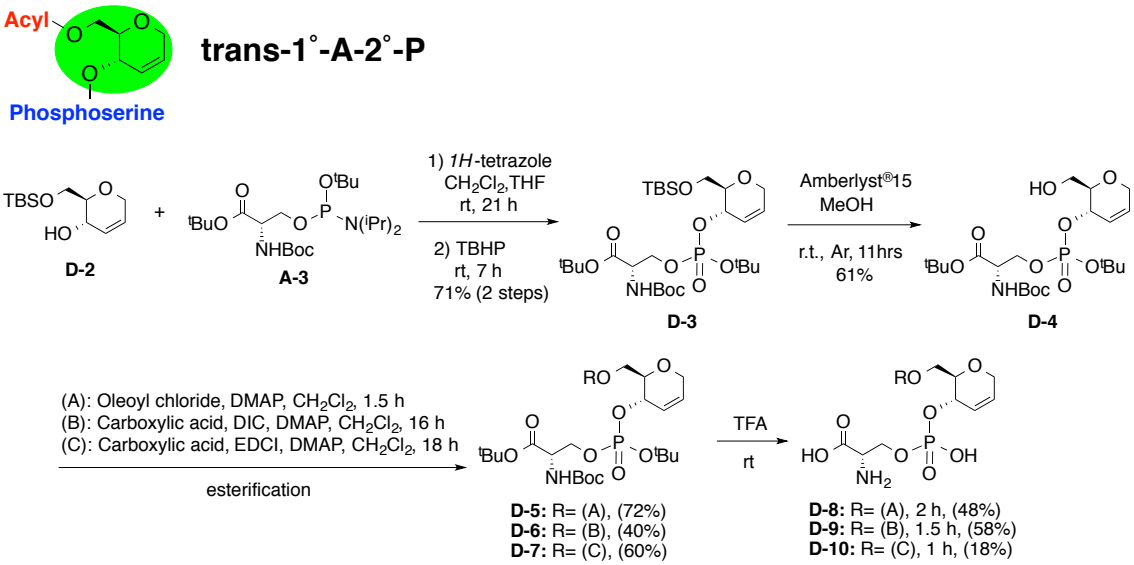


Cf. from **Figure 12**

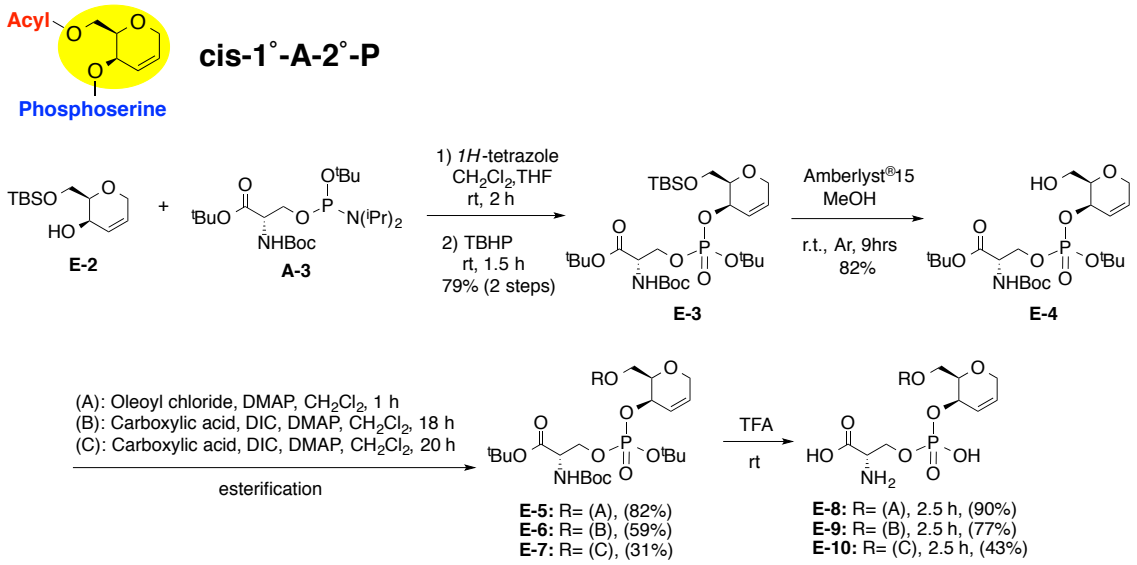
During the chemical modification, I could obtain 3, 6-dihydro-2*H*-pyran moiety as an intermediate by skipping the hydrogenation step for surviving the double bond in the ring to give unsaturated cyclic moiety **D-2** (Scheme 8). **D-2** was connected to the phosphoserine by phosphoramidite method followed by deprotection of silylether to form the primary free alcohol, then esterification. Finally, deprotection was carried out under TFA to afford unsaturated primary acyl compounds (Scheme 9 for trans and Scheme 10 for cis configuration). To obtain the secondary acyl compounds, the synthetic order of connection of phosphoserine unit and esterification can be changed (Scheme 11 for trans and Scheme 12 for cis configuration).



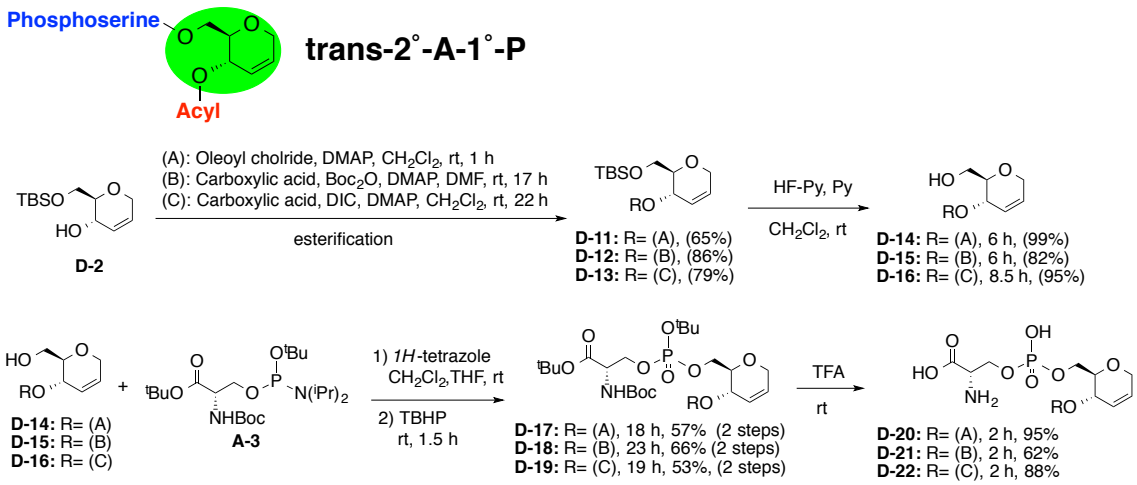
Scheme 8. Synthesis of modified unsaturated cyclic glycerol framework



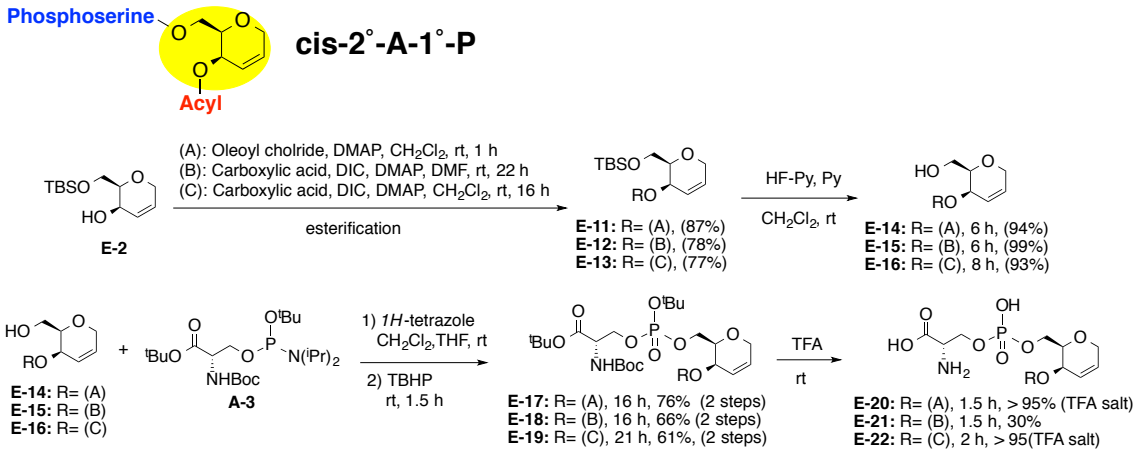
Scheme 9. Synthesis of olefin-trans-1˚-acyl-2˚-phosphoserine



Scheme 10. Synthesis of olefin-cis-1˚-acyl-2˚-phosphoserine



Scheme 11. Synthesis of olefin-trans-2˚-acyl-1˚-phosphoserine



Scheme 12. Synthesis of olefin-cis-2˚-acyl-1˚-phosphoserine

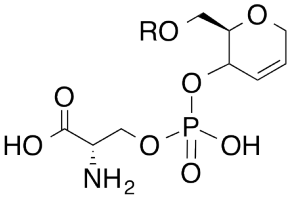
4-4-2. Biological Activities

The synthesized unsaturated cyclic analogues are evaluated their agonistic activities by TGFα shedding assay. The results are shown in Table 5.

The results indicated that acyl chain on the primary position is better for receptor activation also in the case of unsaturated cyclic analgoues regardless of trans/cis and kinds of acyl chain (Table 5). However, one interesting result we got in this study is that trans and cis-secondary acyl compounds containing acyl chain (B), also have quite potent agonistic activity against P2Y10 even though they are still less potent than primary acyl cyclic analogues. We thought that in the case of unsaturated cyclic analogues, they have more restrict glycerol backbone than saturated one. Thus, the effect come from the difference between primary and secondary acyl chain analogues is probably not so significant and sub-type selectivity is appeared considerably.

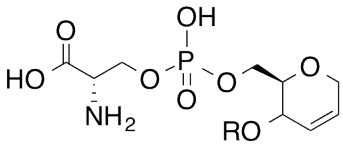
Moreover, we obtained the same results as that of our previous study that acyl chain (B) is the most effective for P2Y10 activation. Especially, compound **D-9** and **E-9** express strong agonistic activity toward P2Y10, 50% and 25% potency of lysoPS respectively.

Table 5. Shedding assay results of oefin-1˚-acyl-2˚-P and oefin-2˚-acyl-1˚-P



D-8: R= (A)
D-9: R= (B)
D-10: R= (C)
E-8: R= (A)
E-9: R= (B)
E-10: R= (C)

Olefin-1˚-acyl-2˚-P

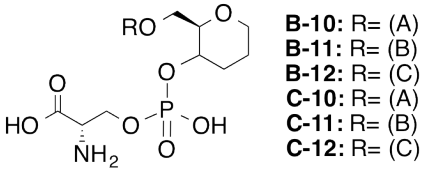


D-20: R= (A)
D-21: R= (B)
D-22: R= (C)
E-20: R= (A)
E-21: R= (B)
E-22: R= (C)

Olefin-2˚-acyl-1˚-P

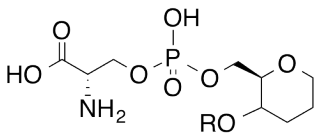
Olefin-*trans*					Olefin-*trans*				
		GPR34	P2Y10	GPR174			GPR34	P2Y10	GPR174
(A)	D-8	255 nM	109 nM	inactive	(A)	D-20	inactive	inactive	inactive
(B)	D-9	inactive	15 nM	inactive	(B)	D-21	inactive	35 nM	inactive
(C)	D-10	180 nM	120 nM	inactive	(C)	D-22	inactive	1080 nM	inactive
Olefin-*cis*					Olefin-*cis*				
		GPR34	P2Y10	GPR174			GPR34	P2Y10	GPR174
(A)	E-8	220 nM	2300 nM	inactive	(A)	E-20	540 nM	inactive	1 μM
(B)	E-9	230 nM	34 nM	inactive	(B)	E-21	inactive	45 nM	>1 μM
(C)	E-10	390 nM	590 nM	inactive	(C)	E-22	36 nM	2883 nM	inactive

Cf. Shedding assay results of saturated-1˚-acyl-2˚-P and 2˚-acyl-1˚-P (from **Table 1**)



B-10: R= (A)
B-11: R= (B)
B-12: R= (C)
C-10: R= (A)
C-11: R= (B)
C-12: R= (C)

1˚-acyl-2˚-P



B-22: R= (A)
B-23: R= (B)
B-24: R= (C)
C-22: R= (A)
C-23: R= (B)
C-24: R= (C)

2˚-acyl-1˚-P

trans					*trans*				
		GPR34	P2Y10	GPR174			GPR34	P2Y10	GPR174
(A)	B-10	130 nM	807 nM	inactive	(A)	B-22	1.6 μM	inactive	inactive
(B)	B-11	>10 μM	106 nM	inactive	(B)	B-23	inactive	inactive	inactive
(C)	B-12	228 nM	6.5 μM	inactive	(C)	B-24	1200 nM	1800 nM	inactive
cis					*cis*				
		GPR34	P2Y10	GPR174			GPR34	P2Y10	GPR174
(A)	C-10	170 nM	inactive	inactive	(A)	C-22	1.6 μM	inactive	inactive
(B)	C-11	200 nM	196 nM	inactive	(B)	C-23	inactive	inactive	inactive
(C)	C-12	54 nM	inactive	inactive	(C)	C-24	98 nM	inactive	inactive

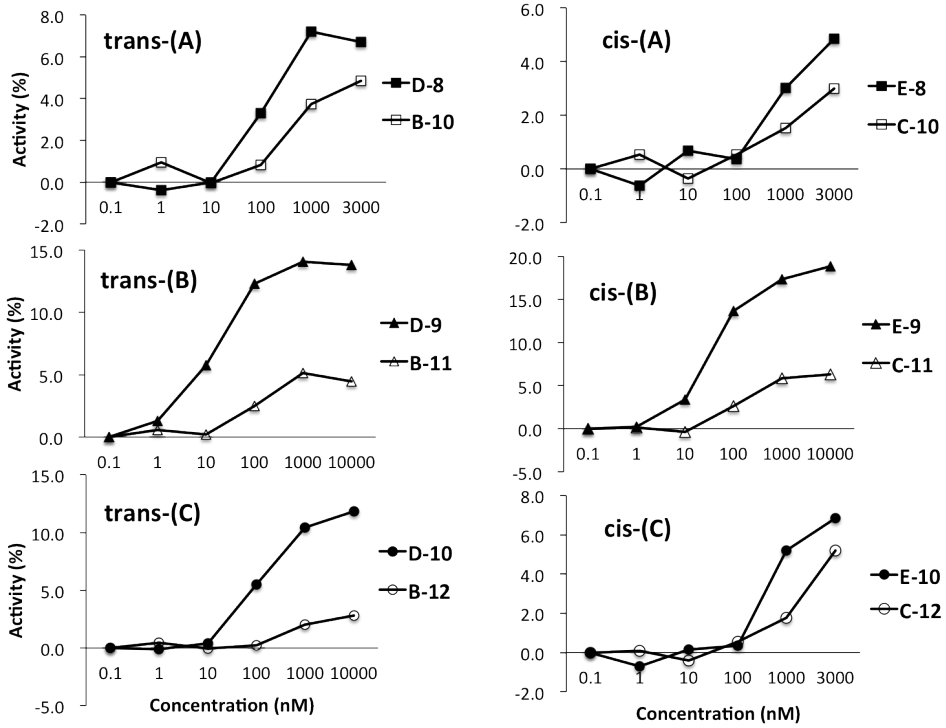


Figure 35. Concentration-response curve of olefin-1˚-acyl-2˚-phosphoserine and 1˚-acyl-2˚-phosphoserine

In addition, it is disclosed that agonistic activity against P2Y10 is increased and GPR34 is decreased as double bond is introduced into the tetrahydropyran ring from the comparison of saturated (Table 1, 1˚-acyl-2˚-P) and unsaturated cyclic analogues (Table 5, olefin-1˚-acyl-2˚-P) (Figure 35). The reason for this result may be the double bond inside of the tetrahydropyran ring, which make the ring planarity increased. Consequently, we thought that planarity of the ring has an influence on the P2Y10 activity.

4-2. Aromatized Glycerol Mimics

The above results encouraged us to study further about the planarity on the tetrahydropyran ring moiety, which might affect to the activity toward P2Y10. Ultimately we replaced the tetrahydropyran framework with a benzene ring, which is more planar than the unsaturated tetrahydropyran, and examined their agonistic activities (Figure 36).

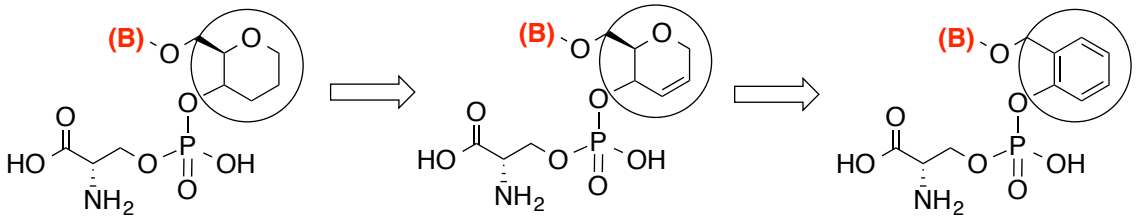


Figure 36. Introduction of benzene ring instead of tetrahydropyran moiety

Considering of the benzene regioisomer, three kinds of benzne-lysoPS analogues, ortho, meta and para, are feasible to be generated (Figure 37).

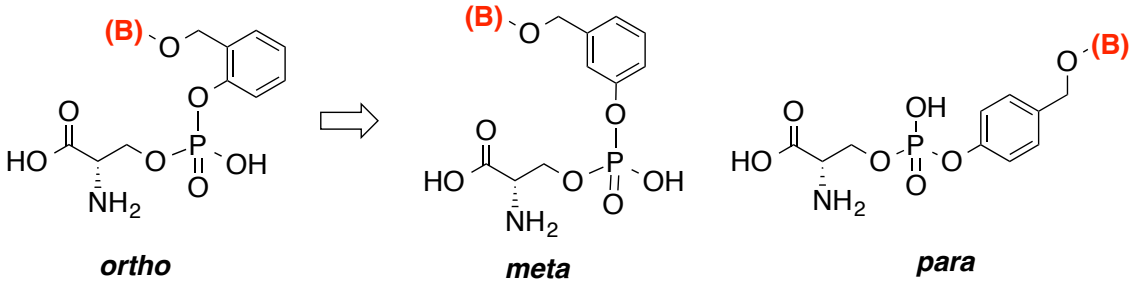
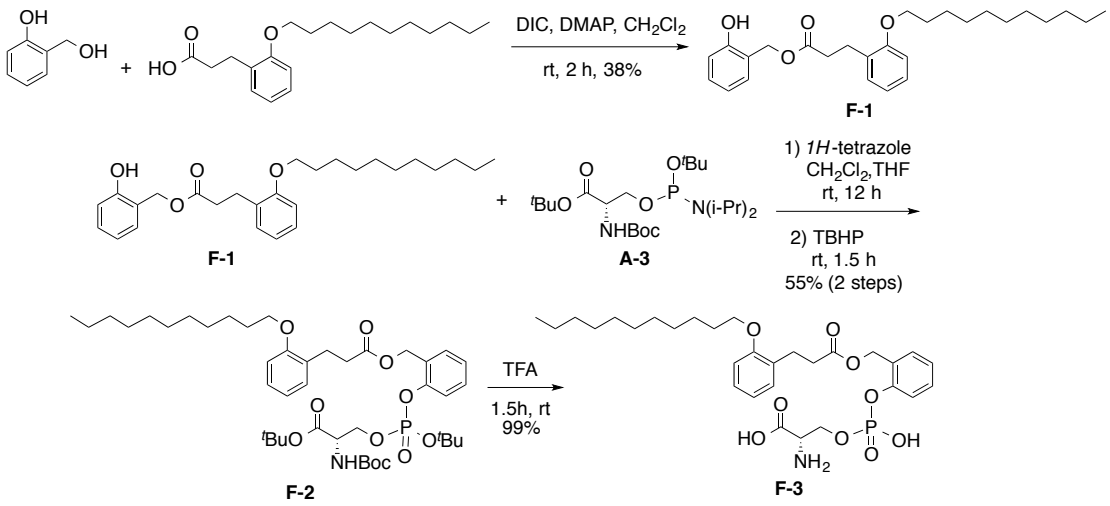


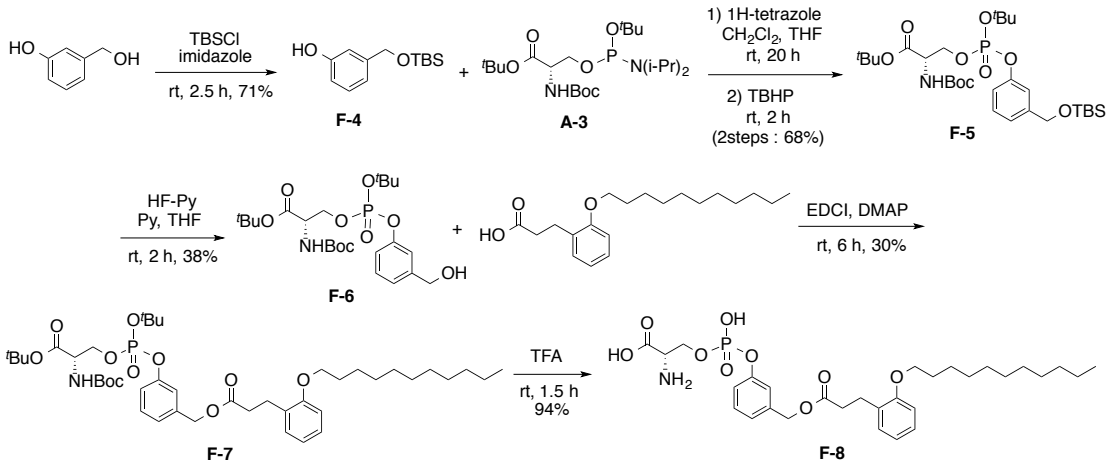
Figure 37. Introducing benzene ring instead of tetrahydropyran moiety

4-2-1. Synthesis of Benzene-LysoPS Analougs

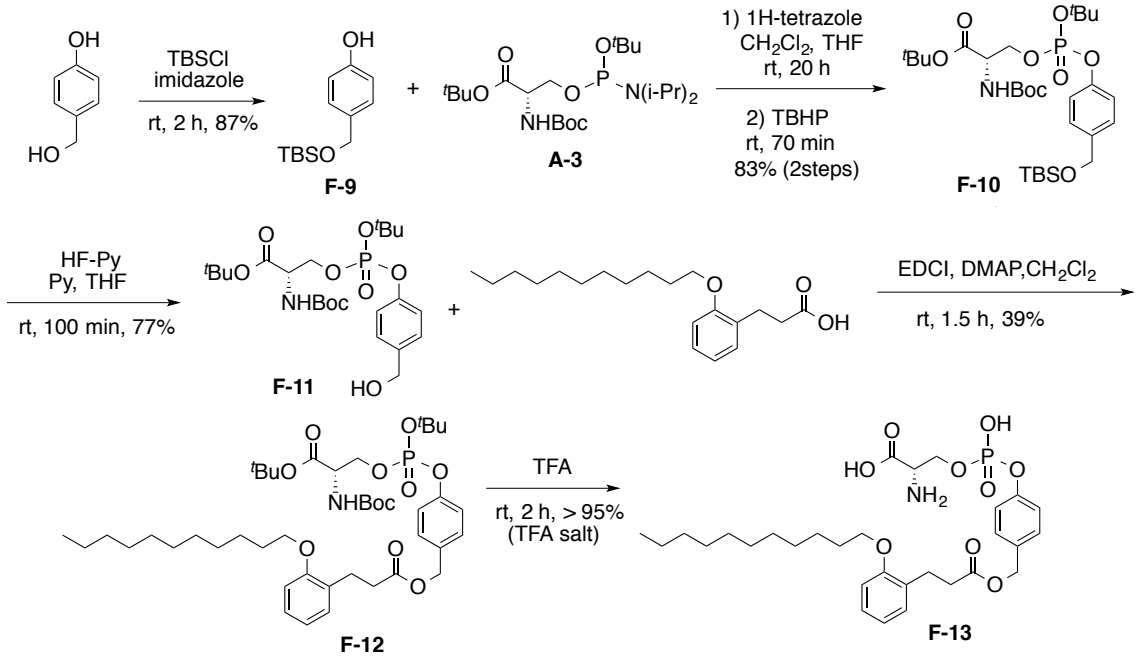
To obtain these benzene-lysoPS analogues, salicylic alcohol (o-hydroxybenzylalcohol), m-hydroxybenzyl alcohol and p-hydroxybenzyl alcohol were used as starting materials. The synthesis was performed through the procedure shown in Schemes 13, 14 and 15: protection of the benzyl alcohol with TBS group and connection of the phenolic oxygen atom to the phosphate group, followed by deprotection of TBS silylether, then esterification of the benzyl alcohol to give protected benzene analogues. Deprotection in TFA gave the aromatic lysoPS derivatives.



Scheme 13. Synthesis of ortho-benzene lysoPS analogue



Scheme 14. Synthesis of meta-benzene lysoPS analogue



Scheme 15. Synthesis of para-benzene lysoPS analogue

4-2-2. Biological Studies

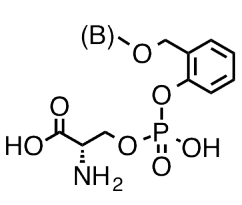
The agonistic activities of these synthesized aromatic lysoPS analogues were evaluated and the results are shown in Table 6.

Subsitution of the glycerol with a benzene ring increased the potency against P2Y10. This result implies that increased planarity has an influence on the potency and selectivity toward GPR34 and P2Y10 by inducing the conformation to be more suitable for activation of P2Y10. We tried to measure dihedral angle at the ring junction in order to confirm whether planarity is involved in the activity changes, and to make sure that planarity is increased with the order from saturated (**C-11**) to unsaturated cyclic analogue (**E-9**) to benzene analouge(**F-13**), which is the same order as the shedding assay results (Figure 38). **C-11** has around -65° to -60°, **E-9** has around 10° samller than **C-11** and benzene analogue has around 0° to 5°. The calculated results indicated that planarity is getting increased from **C-11** to **E-9** to **F-13** and it is clarified that increased potency is corresponded to the planarity increase.

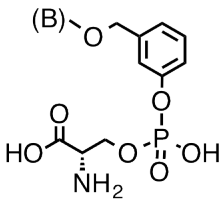
Table 6. Shedding assay results of benzene lysoPS analogues

1-˚-acyl-2˚-phosphoserine

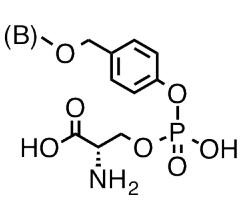
	GPR34	P2Y10	GPR174
ortho (**F-3**)	144 nM	22 nM	inactive
meta (**F-8**)	>1 μM	3.3 nM	2.0 μM
para (**F-13**)	inactive	inactive	inactive



ortho (**F-3**)

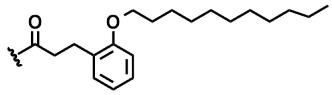


meta (**F-8**)



para (**F-13**)

B)



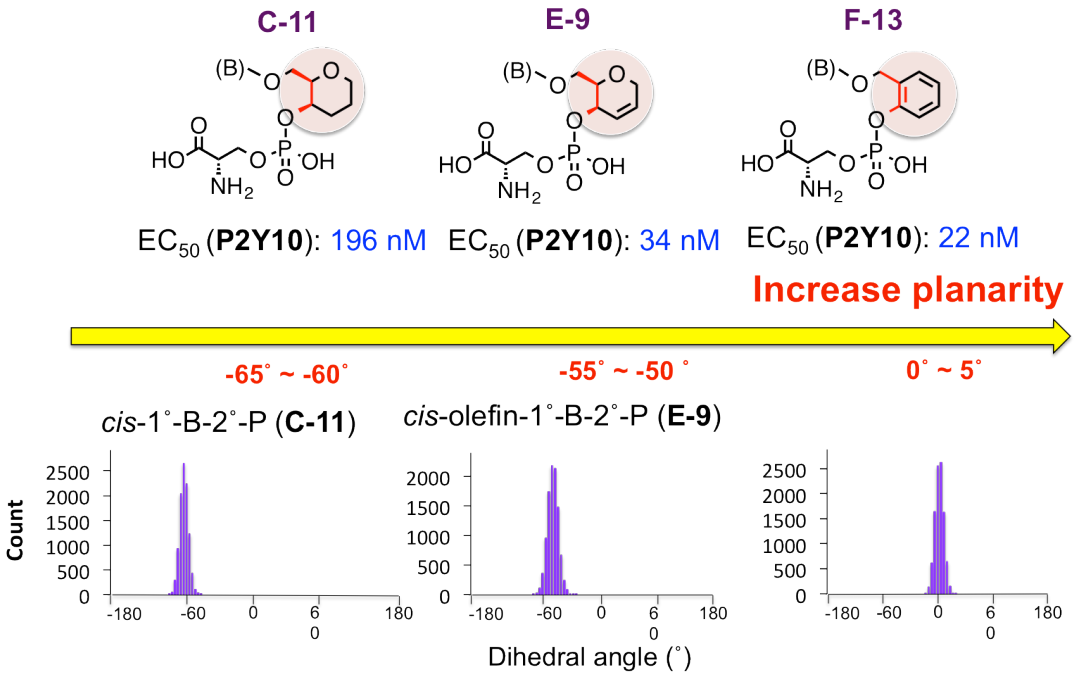


Figure 38. Clarification of affection of increased ring planarity to activity against **P2Y10**

4-3. Conformation Analysis

4-3-1. Examination of General Structure of Cyclic LysoPS Analogues by Measuring Distances and Torsion Angles

We recognized that ***meta*** isomer is the most potent toward P2Y10 comparing to other regioisomers among the benzene lysoPS analogues (Table 6). This means that the substitutied position is also important for agonistic activity to P2Y10. Therefore, I tried to compare the distances and dihedral angles represented in Figure 39 and the results are shown in Table 7 (distances) and Figure 40 (torsion angle).

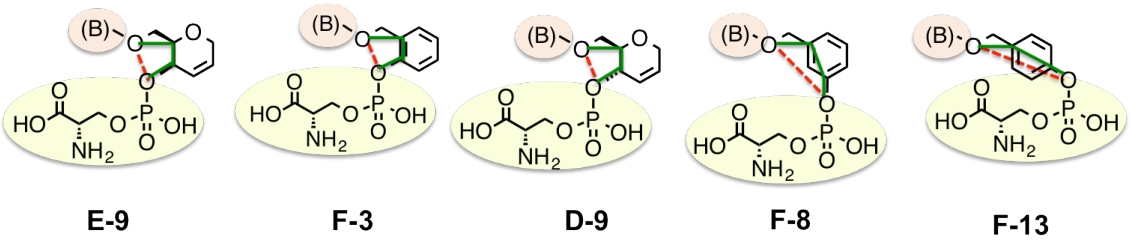


Figure 39. Calculated distances and torsion angles (red; distance, green; torsional angle)

Table 7. Results of calculated distances and biological activities.

	E-9	F-3	D-9	F-8	F-13
EC_{50} **(P2Y10)**	34 nM	22 nM	15 nM	3.3 nM	>10 μM
Distance	4.3 Å	4.0 Å	4.8 Å (4.8 Å)	5.6 Å	6.4 Å

* The calculated initial structure is diequatorial and
the value in the parenthesis is for diaxial conformation.

First of all, *meta*-benzene analogue, **F-8** has around 5.6 Å, which is probably responsible distance of glycerol backbone for the analogue to take accessible conformation toward P2Y10. In the case of *ortho*, **F-3**, it has around 4.0 Å, which is shorter than **F-8**, *para*-benzene analogue, **F-13**, has around 6.4 Å, longer than that of

meta isomer, **F-8**. In the case of **E-9** has longer than **F-3**, and **D-9** has the value between *ortho*, **F-3** and *meta*, **F-8**.

From the results of torsion angle on Figure 40, *meta*-benzene analogue (**F-8**) has high distributions at around -60˚ and 60˚, *ortho*-benzene analogue (**F-3**) also has two major torsion angles near -180˚ and -60˚. In the case of *para*-isomer (**F-13**), the primary acyl chain is freely rotated. For unsaturated cis analogue (**E-9**), has major distribution at aound -120˚ and unsaturated trans anlogue (**D-9**) has near -60˚ as major distribution. From these results, the potent analogues against P2Y10 are likely to take gauche relationship between phosphoserine and acyl chain.

Considering the results of distance, **E-9** is less potent than **F-3** even though it has longer distance than **F-3**. However, its torsion angle is different from the active analogues. This torsion anlge difference may lead to the unfavorable structure of **E-9** and this conformation probably has low simility to the active conformations.

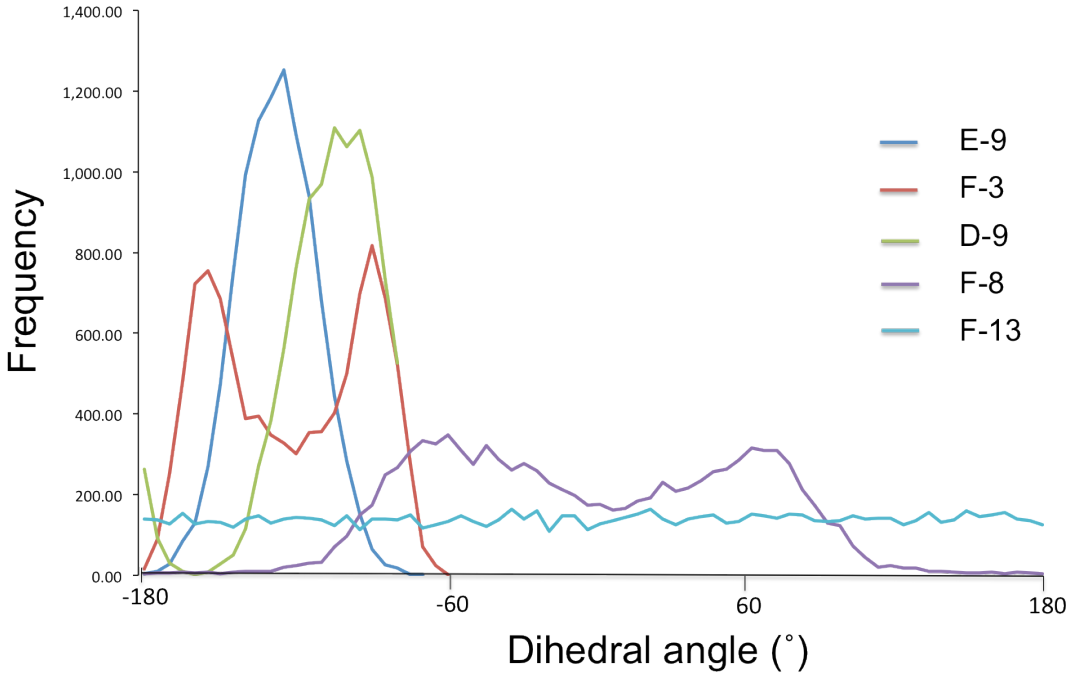


Figure 40. Calculated dihedral angles

Meanwhile, compound **D-9** is more potent than **F-3**, although its planarity is less than **F-3**. To explain this, I considered the two possible conformations of **D-9**, diequatorial and diaxial, and MD simulation was performed twice using two initial structures, diequatorial and diaxial. After MD simulation, I tried to compare dihedral angle at the ring junction between diequatorial and diaxial structure in order to confirm their conformation change during the simulation. The results are shown in Figure 41. It indicates that the conformation of diequatorial initial structure of **D-9** was changed to diaxial during the 100 ns simulation time scale (Figure 41). This means that diaxial structure is more favorable than diequatorial in the case of **D-9**. It is thought that steric hindrance between the large substituents, acyl chain and phosphoserine affect to conformation change of **D-9**. The substituents are likely to avoid the unstability come from the steric hindrance and they are located far from each other. In addition, as taking diaxial structure, 1, 3-diaxial steric hindrance is reduced and leads to more stable conformation.Therefore, the distance of unsaturated trans analogue (**D-9**) is longer than **F-3**, and the planarity on the ring is similar to that of *ortho*-analouge (**F-3**).

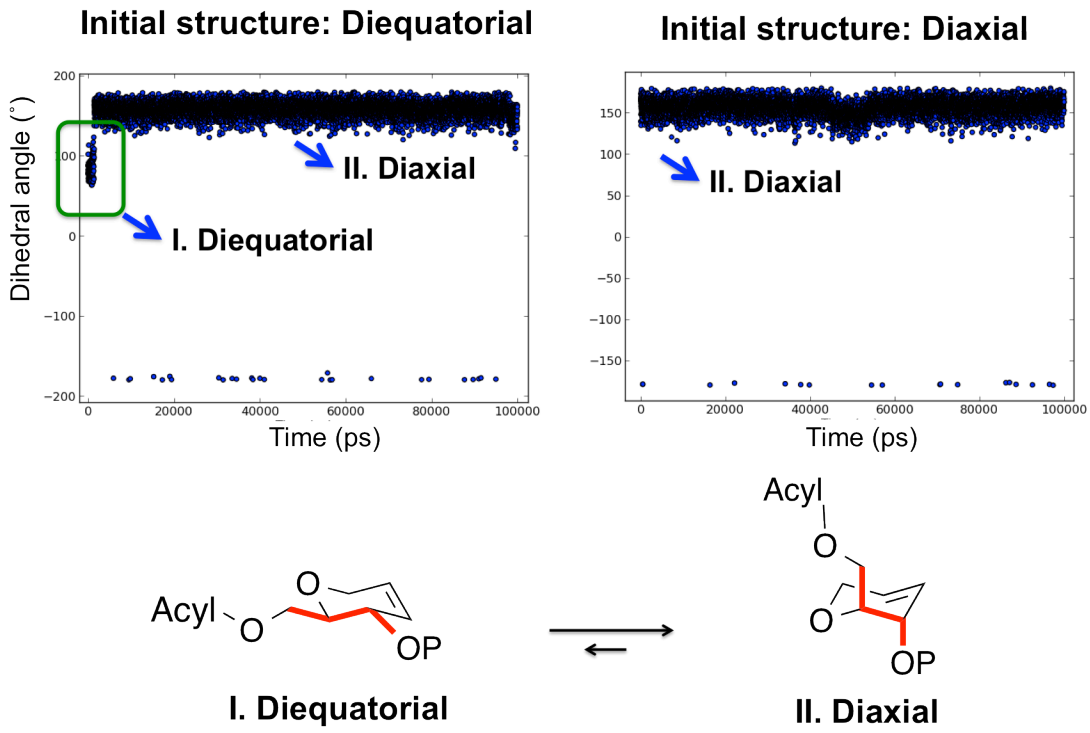


Figure 41. Comparison of dihedral angle diequatorial and diaxial structure of **D-9**

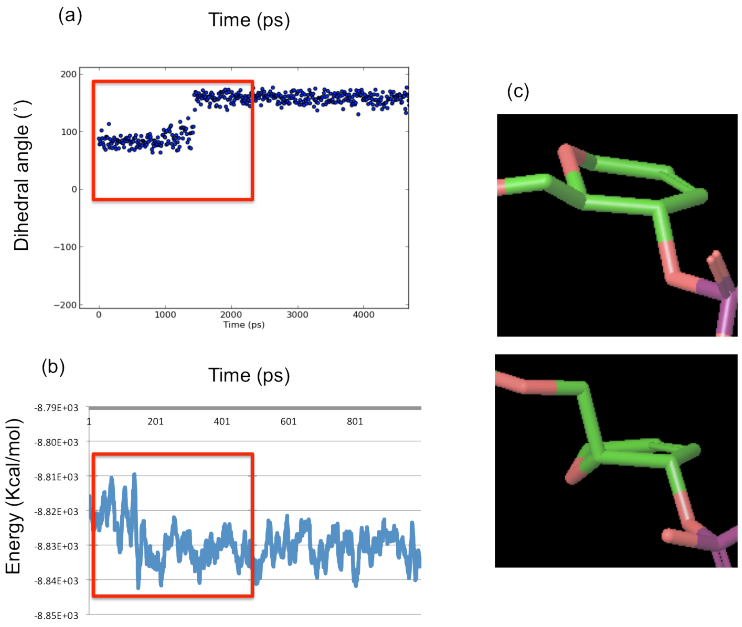


Figure 42. Comparison of dihedral angle and energy change and determination of ring conformations

To support diaxial structure of **D-9** is favorable, I tried to compare the energy of **D-9** depending on the dihedral angle change at the ring junction. The results are shown in Figue 42.

(a) in figure 42 is the graph dihedral angle-simulation time which is expended from 0 to 5 ns time scale. (b) is the graph for energy-simulation time, which is expended from 0 to 8 ns time scale.

The initial structure of **D-9** is diequatorial. Its conformation is quickly changed to diaxial at around 2 ns, and continue to take diaxial conformation to 100 ns. Comparison between dihedral angle (a) and energy change graph (b) shows that dihedral angle change, diequatorial to diaxial, is corresponding to the energy stabilization point (Figure 42). This means that diaxial conformation is more stable than diequatorial. (c) in Figure 42 shows the structure of ring portion, diequtorial structure of **D-9** on the upper picture and diaxial conformation of **D-9** below.

4-3-2. Proposal of LysoPS Conformation Active for P2Y10

Furthermore, we tried to investigate lysoPS conformation for P2Y10 activation comparing with the *meta*-benzene analogue (**F-8**), which is the most potent and selective among the cyclic analogue against P2Y10. From this examination, it is realized that lysoPS, which belong to cluster 2 of previously divided in Figure 32, is well superimposed to **F-8**, especially, glycerol backbone is corresponding to the *meta*-benzene portion greatly (RMSD = 5.0463Å ~8.0308 Å for Cluster1 / 5.2050 Å ~ 7.3857 Å for cluster 2 / 6.7889 Å ~ 9.0513 Å for Cluster3) (Figure 43). This extended glycerol backbone leads to the structure of which acyl chain and phosphoserine are located far from each other and the superimposed image is shown in Figure 44.

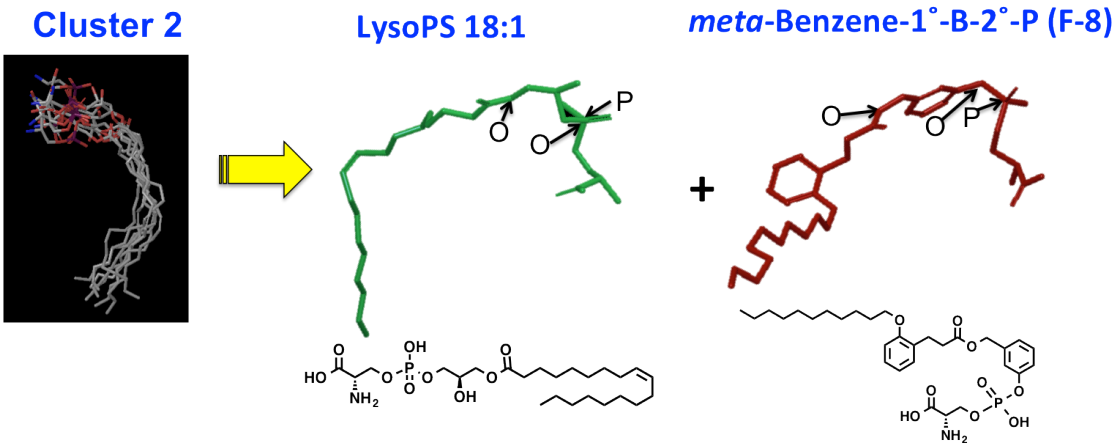


Figure 43. Comparison of lysoPS to the benzene lysoPS analogue **F-8**

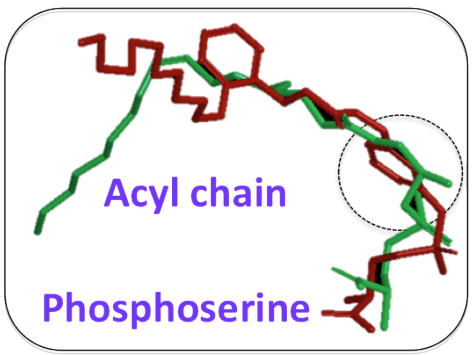


Figure 44. Superimposed image of **F-8** and lysoPS

Chapter 5. Conclusion

We developed novel conformationally constrained lysophosphatidylserine analogues, which are potent and selective against GPR34 and P2Y10. We expected that they could be utilized as useful tools for further biological assay study to control selective GPR34 and P2Y10. Moreover, it is predicted that these cyclic analogues provide metabolically more stabilized lysoPS in that they are the analogues, which have eliminated hydroxyl group function of glycerol backbone, which is involved in destabilization of lysoPS by enzymatical hydrolysis and acyl migration etc.

In this study, we successfully established the synthetic route to obtain 1˚-acyl-2˚-phosphoserine and 2˚-acyl-1˚-phosphoserine analogues by introducing tetrahydropyran moiety, which has non-equivalent hydroxyl group to afford diversified cyclic lysoPS analogues for conformation study. In addition, it is revealed that 1˚-acyl-2˚-phosphoserine analogues are more effective for receptor activation and disclosed active conformations by trans and cis configuration toward each receptor by calculation study.

Finally, we suggested that active conformations of lysoPS toward GPR34 and P2Y10 by comparing selectively potent cyclic lysoPS analogues and lysoPS itself on the basis of taking similar conformation between lysoPS and selective GPR34 and P2Y10 agonists. These results are predicted to provide useful tools for searching for phormacophore points and modeling and docking study for clarification of three dimenssional GPR34 and P2Y10 structure hereafter.

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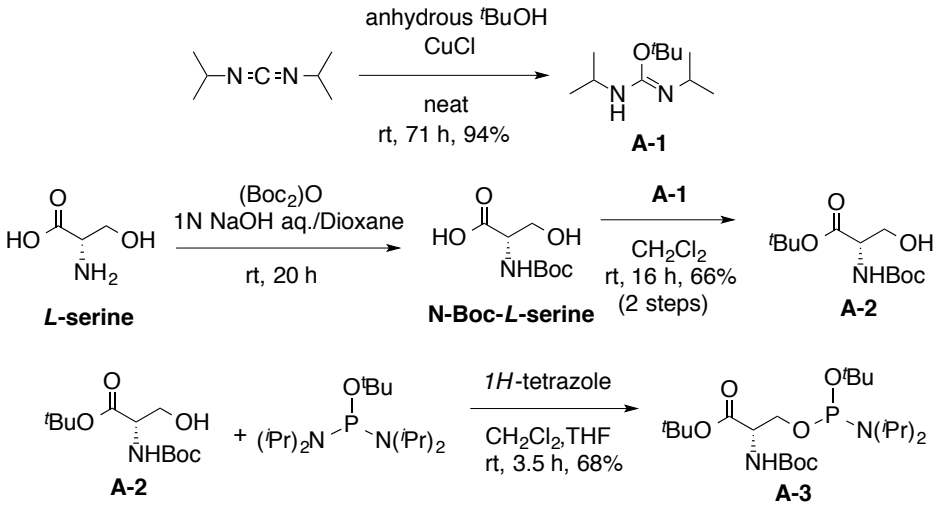
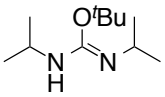
Experimental Section

1. Synthesis of phosphoserine unit

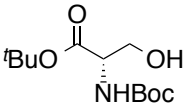


Compound **A-1**



1,3-Diisopropylcarbodiimide (6.003 g, 47.57 mmol) and CuCl (0.047 g, 0.48 mmol) was added to a two-neck flask and the vessel was exchanged with Ar. ^{t}BuOH (4.230 g, 57.08 mmol) was added to the reaction mixture and the whole was stirred at room temperature under Ar atmosphere for 3 days. A small amount of the reaction mixture was withdrawn some amount of the reaction mixture to confirm whether the reaction was completed. CH_2Cl_2 (24 ml) and polyvinyl pyridine (0.950 g) were added to the reaction mixture and the whole was stirred at room temperature for 15min. The whole was filtered on celite and the filtrate was evaporated to remove the solvent to give **A-1** (8.927 g, 44.56 mmol, 94 %, green oil).
^{1}H NMR ($CDCl_3$): δ= 3.733-3.610 (1H, m), 3.225-3.095 (1H, m), 1.456-1.029 (21H, m).

Compound **A-2**

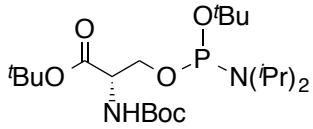


L-serine (1.025 g, 9.756 mmol) was dissolved in 1N aqueous NaOH solution (10 ml), H_2O (10 ml), and dioxane (20 ml) and the whole was 0℃ with stirring. Di-*tert*-butyl dicarbonate (3.192 g, 14.63 mmol) was added to the solution at 0°C with stirring over 10 min and the reaction mixture was stirred at room temperature for 20 h. 5 % aqueous $KHSO_4$ was added to the reaction mixture to be acidified at pH 3. The solution was extracted with ethyl acetate (150

ml× 3). The combined organic layer was washed with brine, dried over $MgSO_4$, and the solvent was evaporated to yield crude **A-2** (2.228 g, crude colorless oil).

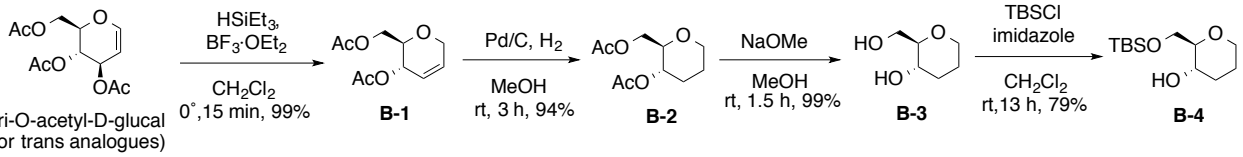
Crude compound **A-2** (2.228 g) was dissolved in CH_2Cl_2 (150 ml) and **A-1** (7.112 g, 35.50 mmol) was added to the solution above and the reaction mixture was stirred at room temperature for 16 hours. Hexane (150 ml) was added to the reaction mixture and the whole was stirred for 10 min. The reaction mixture was filtered on celite and the filtrate was evaporated. The residue was purified by column chromatography (hexane : ethyl acetate = 3:1) to yield **A-2** (1.677 g, 6.418 mmol, 66 % (2 steps), white solid).
^{1}H NMR ($CDCl_3$): δ= 5.407 (1H, m), 4.255 (1H, m), 3.897 (2H, m), 2.333 (1H, brs), 1.483 (9H, s), 1.452 (9H, s).
HRMS (ESI-TOF [M+Na]$^+$): Calcd. for $C_{12}H_{23}NNaO_5^+$: 284.1468. Found: 284.1469.
Anal. Calcd. for: C, 55.16; H, 8.78; N, 5.36. Found: C, 55.16; H, 8.78; N, 5.36.

Compound **A-3**

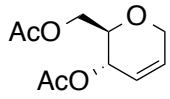


Bis(diisopropylamino)tert-butylphosphine (1.0 g, 3.83 mmol) was dissolved in CH_2Cl_2 (15 ml) and toluene (1.5 ml). Compound **A-2** (1.301 g, 4.979 mmol) was added to the solution and the solvent was evaporated to remove containing water. The residue was dissolved in dry CH_2Cl_2 (15ml) under Ar atmosphere and 1*H*-tetrazole in THF (15ml) was added to the solution at room temperature. The reaction mixture was stirred for 3.5 hours. After 3.5 hours, the reaction was quenched with saturated aqueous $NaHCO_3$ (20 ml) and the whole was extracted with CH_2Cl_2 (20 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 : Et_3N = 35 : 4 : 1) to yield **8** (1.56 g, 3.36 mmol, 67.5 %, yellow oil).
^{1}H NMR (CD_2Cl_2): δ= 5.431 (1/2H, d, *J*= 6.3Hz), 5.260 (1/2H, d, *J*= 6.3Hz), 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): δ= 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): δ= 138.92, 138.44.
HRMS(ESI-TOF[M+Na]$^+$): Calcd. for $C_{22}H_{45}N_2NaO_6P^+$: 487.2907, Found : 487.2906.
Anal. Calcd. for: C, 56.88; H, 9.76; N, 6.03. Found: C, 55.94; H, 9.72; N, 5.86.

2. Synthesis of modified glycerol backbone (trans)

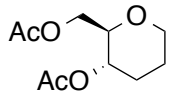


Compound **B-1**



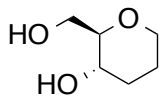
Tri-O-acetyl-D-glucal (1.0003 g, 3.6731 mmol) and triethylsilane (512.53 mg, 4.41 mmol) were dissolved in CH_2Cl_2 (5 mL) at room temperature under Ar. The solution was cooled to 0 ℃. $BF_3{\cdot}OEt_2$ (521.32 mg, 3.67 mmol) was added dropwise to the solution above at 0 ℃ under Ar atmosphere. The reaction mixture was stirred 0 ℃ 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 mL×2), brine (5 mL), and then dried over $MgSO_4$. Combined organic layer was evaporated to yield **B-1** (784.6 mg, 3.66 mmol, 99.71 %, colorless oil).
^{1}H NMR ($CDCl_3$): δ= 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 ($CDCl_3$): δ= 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, 54.14; H, 6.66; N, 0.00; ($H_2OX0.2$). Found: C, 55.17; H, 6.45; N, 5.86.

Compound **B-2**



Compound **B-1** (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 **B-2** (2.0224 mg, 9.3530 mmol, 93.72 %, colorless oil).
^{1}H NMR ($CDCl_3$): δ= 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 ($CDCl_3$): δ= 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: 239.0934
Anal. Calcd. for C, 53.98; H, 7.27; N, 0.00; ($CH_2Cl_2X0.1$). Found: C, 54.14 H, 7.24; N, 0.00.

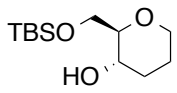
Compound **B-3**



Compound **B-2** (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. The reaction mixture was stirred at room temperature under Ar for 1.5 hrs. After 1.5 hrs, solvent was removed and the

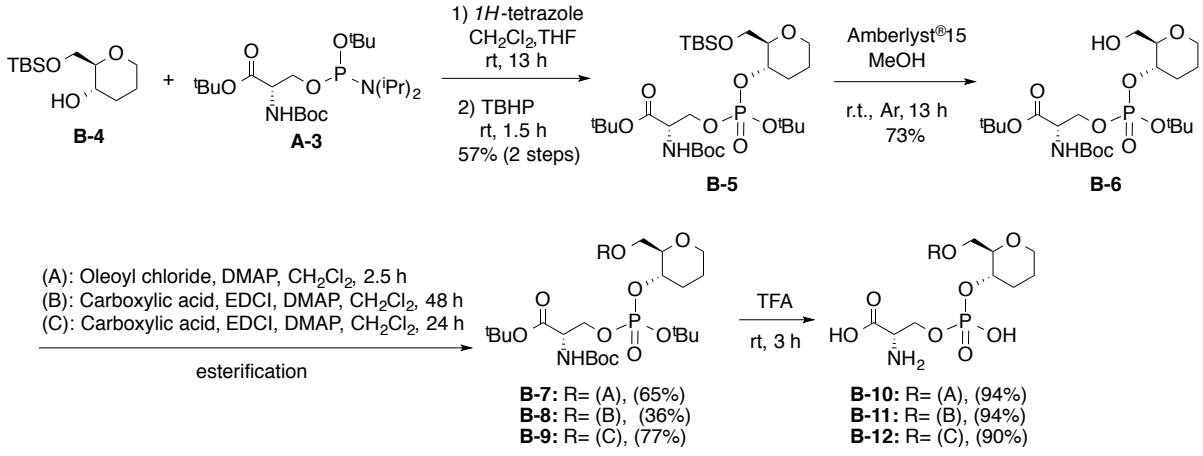
residue was dissolved in $CHCl_3$. This solution was filtered on celite and the filtrate was evaporated to yield **B-3** (1.2031 mg, 9.1034 mmol, 99.3 %, colorless oil).
^{1}-H NMR ($CDCl_3$): δ= 3.931-3.901 (1H, m), 3.853-3.751 (2H, m), 3.584-3.522(1H, m), 3.397-3.332 (1H, m), 3.150-3.105 (1H, m), 2.598 (2H, brs), 2.134-2.083 (1H, m), 1.710-1.643 (2H, m), 1.483-1.381 (1H, m).
^{13}-C NMR ($CDCl_3$): δ= 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.

Compound **B-4**

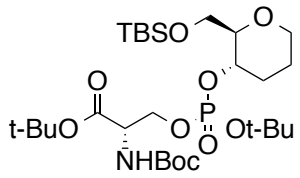


Compound **B-3** (24.0 mg, 0.1816 mmol) and imidazole (27.2 mg, 0.3995 mmol) was dissolved in DMF (0.25 mL) and cooled to 0 ℃. *t*-Butyldimethylchloro silane (32.8 mg, 0.2179 mmol) was added to the solution above at 0 ℃ and the reaction mixture was stirred at room temperature under Ar for 13 hrs. After 13 hrs, 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 mL×2), brine (2 mL) and dried over $MgSO_4$, then evaporated. The residue was purified by column chromatography to yield **B-4** (35.5 mg, 0.1441 mmol, 79.3 %, colorless oil).
^{1}-H NMR ($CDCl_3$): δ= 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, 2.0Hz), 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 ($CDCl_3$): δ= 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, 56.31; H, 10.23; N, 0.00; (CH_2Cl_2X0.15). Found: C, 56.50 H, 10.04; N, 0.00.

3. Synthesis of trans-1˚-acyl-2˚-phosphoserine

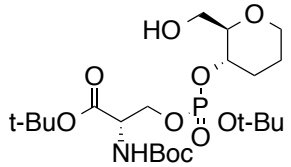


Compound **B-5**



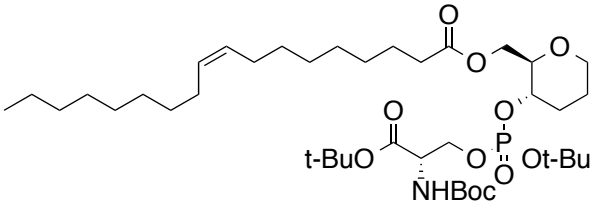
A-3 (338.0 mg, 0.7486 mmol) was dissolved in CH_2Cl_2 (5 mL) and toluene (0.5 mL) and co-evaporated to remove containing water. Compound **B-4** (276.7 mg, 1.1229 mmol) was added to the solution above and dissolved in CH_2Cl_2 (5 mL) and toluene (0.5 mL) then, co-evaporated. The residue was dissolved in dry CH_2Cl_2 (5 mL) and 1H-tetrazole (209.7 mg, 2.9939 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 hrs. TBHP was added to the reaction mixture and stirred at room temperature under Ar for 1.5 hrs. After 1.5 hrs, the reaction mixture was quenched with H_2O (10 mL) and extracted with CH_2Cl_2 (5 mL×3). The organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography (hexane : ethyl acetate = 4:1 to 2:1) to yield **B-5** (397.1 mg, 0.6345 mmol, 84.76 %, colorless oil (56.51 %, 2 steps)).
^{1}H NMR ($CDCl_3$): δ= 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 ($CDCl_3$): δ= 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 ($CDCl_3$): δ= -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, 53.74; H, 9.02; N, 2.24. Found C, 53.71; H, 9.01; N, 2.32.

Compound **B-6**



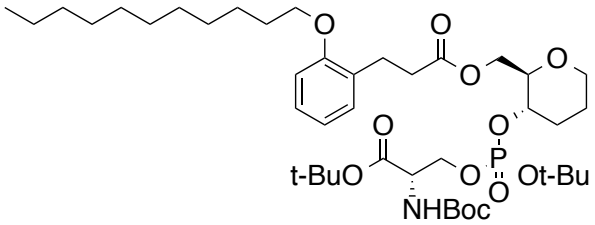
Compound **B-5** (203.1 mg, 0.3245 mmol) was dissolved in MeOH (8 mL) and cooled to 0 ℃. Amberlyst®15 (1.0155 g) was added to the solution above and the reaction mixture was stirred at 0 ℃ under Ar atmosphere for 10 min. After 15 min, the reaction mixture was stirred at room temperature under Ar atmosphere for 13 hrs. After 13 hrs, the reaction mixture was filtered on celite and the filtrate was evaporated and the residue was purified by column chromatography (ethyl acetate) to yield **B-6** (121.3 mg, 0.2371 mmol, 73.07 %, white sticky solid).
^{1}H NMR ($CDCl_3$): δ= 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 ($CDCl_3$): δ= 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 ($CDCl_3$): δ= -5.18, -5.33.
HRMS (ESI-TOF $[M+Na]^+$): Calcd. for $C_{22}H_{42}NNaO_{10}P^+$: 534.2439, Found 534.2391.

Compound **B-7**



Compound **B-6** (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 and stirred at room temperature under Ar atmosphere for 2.5 hrs. 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 (hexane : ethyl acetate = 2:1) to yield **B-7** (120.9 mg, 0.1558 mmol, 65.0 %, translucent oil).
^{1}H NMR ($CDCl_3$): δ= 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 ($CDCl_3$): δ= 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 ($CDCl_3$): δ= -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, 60.92; H, 9.40; N, 1.77; (CH_2Cl_2X0.2). Found C, 61.14; H, 9.12; N, 1.82.

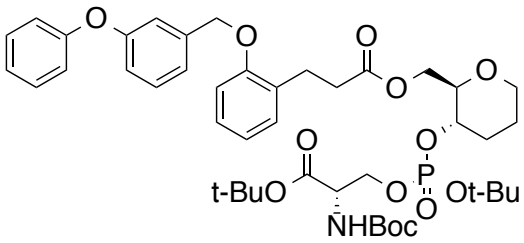
Compound **B-8**



Compound **B-6** (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. Reaction mixture was stirred at room temperature under Ar atmosphere for 48 hrs. EDCI (1.3 eq) and MeOH (1 mL) were added to the reaction mixture and stirred at room temperature for 2 hrs. 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 (hexane : ethyl acetate = 2:1) to yield **B-8** (53.2 mg, 0.0654 mmol, 35.6 %, colorless oil)
^{1}H NMR ($CDCl_3$): δ= 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

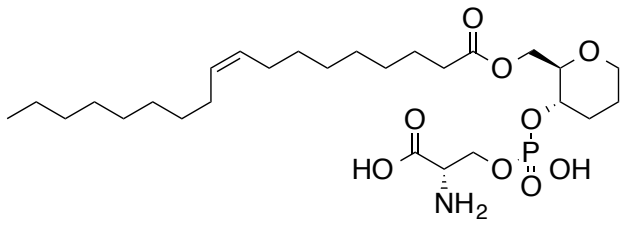
(1H, m), 4.154-4.047 (2H, m), 3.950-3.904 (3H, m), 3.393-3.307 (2H, m), 2.957-2.918 (2H, m), 2.376-2.345 (2H, m), 1.798-1.568 (5H, m), 1.488-1.259 (43H, m), 0.887-0.853 (3H, m).
^{13}-C NMR ($CDCl_3$): δ= 173.22, 173.20, 168.30, 156.96, 155.32, 155.21, 129.95, 129.01, 128.96, 127.40, 127.38, 120.12, 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 ($CDCl_3$): δ= -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, 61.97; H, 8.92; N, 1.72. Found C, 61.70; H, 8.68; N, 1.74.

Compound **B-9**



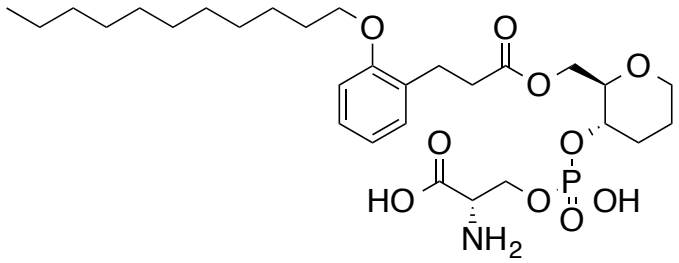
Compound **B-6** (54.4 mg, 0.1063 mmol) and carboxylic acid derivative (**C**) (48.2 mg, 0.1382 mmol) were dissolved in CH_2Cl_2 (2 mL). EDCI (26.5 mg, 0.1382 mmol) and DMAP (1.7 mg, 0.0138 mmol) were added to the solution above. Reaction mixture was stirred at room temperature under Ar atmosphere for 24 hrs. EDCI (0.3 eq) and MeOH (1 mL) were added to the reaction mixture and stirred at room temperature for 1hrs. H_2O (5 mL) was added to the reaction mixture and extracted with CH_2Cl_2 (3 mL× 3). Organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography (hexane : ethyl acetate = 2:1 to 1:1) to yield **B-9** (69.1 mg, 0.0821 mmol, 77.21 %, colorless oil).
^{1}-H NMR ($CDCl_3$): δ= 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 ($CDCl_3$): δ= 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 ($CDCl_3$): δ= -6.28, -6.33.
HRMS (ESI-TOF [M+Na]$^+$): Calcd. for $C_{44}H_{60}NNaO_{13}P^+$: 864.3694, Found 864.3689.

Compound **B-10**



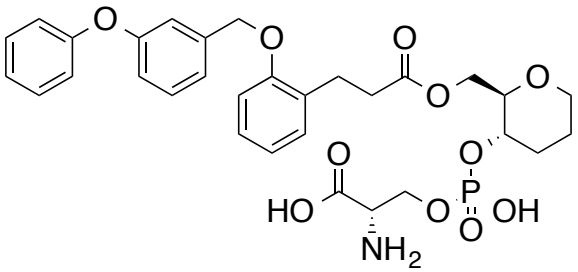
Compound **B-7** (111.2 mg, 0.1433 mmol) was dissolved in trifluoroacetic acid at 0 ℃ and stirred at 0 ℃ for 30 min. After 30 min, solvent was evaporated under vacuum and the residue was purified by column chromatography $CHCl_3$: MeOH : AcOH = 8:1:1) to yield the compound which had one *tert*-butyl group in its molecule (103.1 mg). The obtained compound was dissolved in trifluoroacetic acid at 0 ℃ and stirred at room temperature for 2.5 hrs. After 2.5 hrs, 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 **B-10** (75.9 mg, 0.1347 mmol, 94.0 %, white solid).
^{1}H NMR ($CDCl_3$): δ= 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 ($CDCl_3$): δ= -3.10.
HRMS (ESI-TOF: $[M-H]^-$): Calcd. for $C_{27}H_{49}NO_9P^-$: 562.3150, Found 562.3178.
Anal. Calcd. for C, 52.46; H, 7.77; N, 2.14; ($CF_3CO_2H\times0.8$). Found C, 52.20; H, 7.72; N, 2.16.

Compound **B-11**



Compound **B-8** was dissolved in trifluoroacetic acid at 0 ℃. This was stirred at 0 ℃ for 10 min. After 10 min, the reaction mixture was stirred at room temperature for 3 hrs. After 3 hrs, solvent was removed and the residue was purified by column chromatography ($CHCl_3$: MeOH : AcOH = 8:1:1 to 6:1:2) to yield **B-11** (43.6 mg, 0.0725 mmol, 94.4 %, white soild).
^{1}H NMR ($CDCl_3$): δ= 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 ($CDCl_3$): δ= -2.90.
HRMS (ESI-TOF: $[M-H]^-$): Calcd. for $C_{29}H_{47}NO_{10}P^-$: 600.2943, Found 600.2934.
Anal. Calcd. for C, 52.02; H, 6.90; N, 1.96. Found C, 52.06; H, 7.01; N, 1.91.

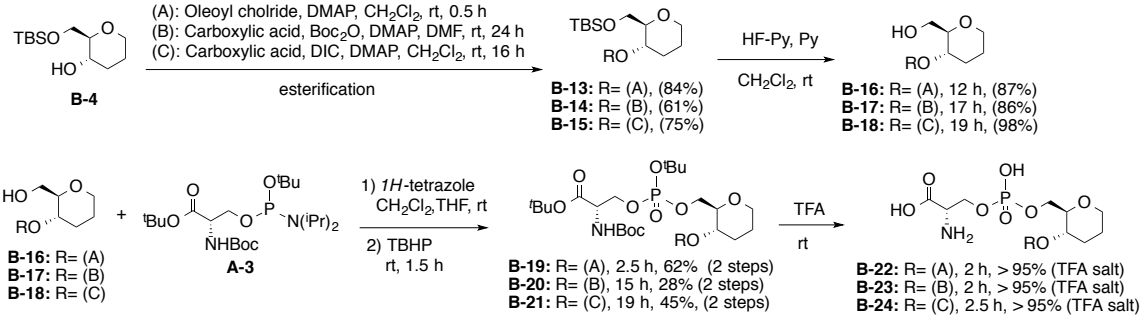
Compound **B-12**



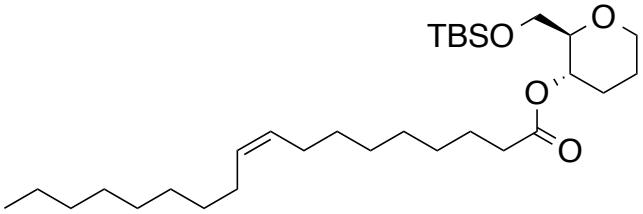
Compound **B-9** was dissolved in TFA at 0 ℃ and stirred at 0 ℃ for 10 min. After 10 min, the reaction mixture was stirred at room temperature for 2.5 hrs. After 2.5 hrs, the reaction

mixture was evaporated and the residue was purified by column chromatography ($CHCl_3$: MeOH : AcOH=8:1:1 to 6:1:3) to yield **B-12** (47.3 mg, 0.0751 mmol, 90.1 %, white solid).
^{1}H NMR ($CDCl_3$): δ= 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 ($CDCl_3$): δ= -2.73.
HRMS (ESI-TOF: $[M-H]^-$): Calcd. for $C_{31}H_{35}NO_{11}P^-$: 628.1953, Found 628.1968.
Anal. Calcd. for C, 50.17; H, 4.58; N, 1.70. Found C, 50.07; H, 4.95; N, 1.60.

4. Synthesis of trans-2˚-acyl-1˚-phosphoserine

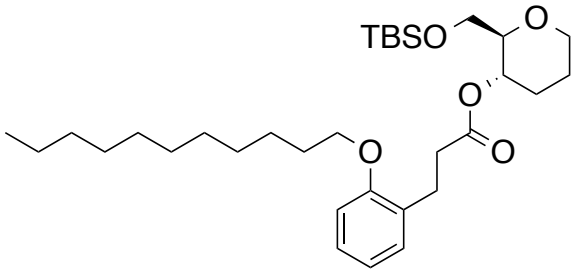


Compound **B-13**



Compound **B-4** (100.0 mg, 0.4058 mmol) was dissolved in CH_2Cl_2 (2 mL), *N*,*N*-dimethylaminopyridine (148.7 mg, 1.2174 mmol) was added to the solution and the mixture was cooled to 0 ℃. Oleoyl chloride (183.2 mg, 0.6087 mmol) was added to the solution above and stirred at 0 ℃ under Ar for 30 min. After 30 min, the reaction mixture was evaporated under vacuum to remove solvent and the residue was purified by column chromatography (hexane : ethyl acetate = 8:1) to yield **B-13** (174.8 mg, 0.3422 mmol, 84.32 %, colorless oil).
^{1}H NMR ($CDCl_3$): δ= 5.411-5.289 (2H, m), 4.699-4.646 (1H, m), 3.937-3.925 (1H, m), 3.719 (1H, dd, *J*= 11.6 Hz, 2.4 Hz), 3.656 (1H, dd, *J*= 11.6 Hz, 5.3 Hz), 3.392-3.356 (1H, m), 3.334-3.305 (1H, m), 2.277-2.164 (3H, m), 2.053-1.982 (4H, m), 1.758-1.562 (4H, m), 1.468-1.252 (21H, m), 0.891-0.859 (12H, m), 0.045-0.041 (6H, m).
^{13} C NMR ($CDCl_3$): δ= 172.71, 130.00, 129.72, 80.61, 68.23, 67.63, 63.44, 34.55, 31.90, 31.52, 29.76, 29.70, 29.60, 29.52, 29.33, 29.25, 29.17, 29.10, 27.22, 27.16, 25.99, 24.99, 24.96, 22.68, 18.47, 14.11, -5.27, -5.30.
HRMS (ESI-TOF $[M+Na]^+$): Calcd. for $C_{30}H_{58}NaO_4Si^+$: 533.3997, Found 533.3999.

Compound **B-14**



Compound **B-4** (101.2 mg, 0.4107 mmol), carboxylic acid derivatives **(B)** (101.2 mg, 0.3159 mmol), di-*tert*-butyl dicarbonate (89.6 mg, 0.4107 mmol) and *N,N*-dimethylaminopyridine (2 mg, 0.0158 mmol) were dissolved in DMF (1.5 mL) and stirred at room temperature under Ar atmosphere for 15 hrs. After 15 hrs, the reaction mixture was heated to 40 ℃ and stirred under Ar for 24 hrs. The reaction mixture was diluted with ethyl acetate (6 mL) and washed with saturated aqueous NH_4Cl solution (5 mL), $NaHCO_3$ (5 mL× 2), brine, dried over $MgSO_4$ and evaporated sequentially. The residue was purified by column chromatography (hexane : ethyl acetate = 20:1) to yield **B-14** (138.5 mg, 0.2523 mmol, 61.44 %, colorless oil).

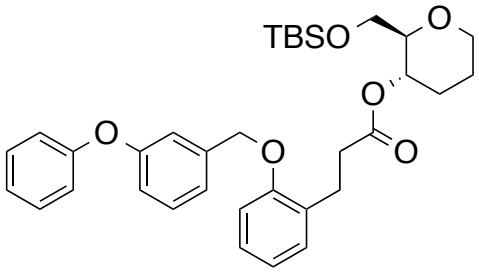
^{1}-H NMR ($CDCl_3$): δ= 7.189-7.125 (2H, m), 6.871-6.811 (2H, m), 4.715-4.652 (1H, m), 3.978-3.933 (3H, m), 3.684-3.618 (2H, m), 3.369 (1H, dd, *J*= 11.6 Hz, 2.4 Hz), 3.328-3.273 (1H, m), 2.950-2.911 (2H, m), 2.614-2.575 (2H, m), 2.218-2.179 (1H, m), 1.784-1.613 (3H, m), 1.486-1.280 (18H, m), 0.904-0.873 (12H, m), 0.065-0.040 (6H, m).

^{13}-C NMR ($CDCl_3$): δ= 172.29, 152.60, 129.94, 128.78, 127.55, 120.18, 111.0080.62, 68.28, 67.72, 67.64, 63.37,34.39, 31.93, 29.67, 29.65, 29.41, 29.36, 29.33, 27.78, 26.27, 26.18, 27.02, 26.00, 25.97, 24.98, 22.69, 18.47, 14.12.

HRMS (ESI-TOF $[M+Na]^+$): Calcd. for $C_{32}H_{56}NaO_5Si^+$: 571.3789, Found 571.3787.

Anal. Calcd. for C, 69.17; H, 10.16; N, 0.00; (CH_2Cl_2X0.1). Found C, 69.36; H, 9,76; N, 0.00.

Compound **B-15**

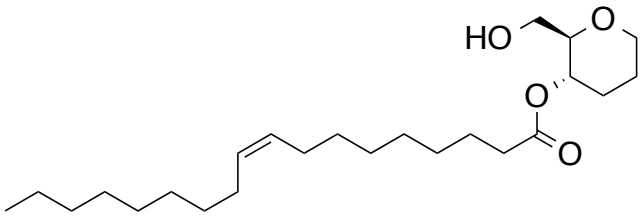


Carboxylic acid derivative (**C**) (260.2 mg, 0.7469 mmol) was dissolved in CH_2Cl_2 (2 mL), diisopropylcarbodiimide (122.5 mg, 0.9709 mmol) and *N,N*-dimethylaminopyridine (9 mg, 0.0747 mmol) was added to the solution above. Compound **B-4** (184.0 mg, 0.7469 mmol) was added to the reaction mixture and the whole was stirred at room temperature under Ar atmosphere for 16 hrs. After 16 hrs, the reaction mixture was diluted with CH_2Cl_2 (7 mL) and quenched by water (7 mL). Water layer was 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 (hexane : ethyl acetate = 15:1 to 8:1) to yield **B-15** (324.5 mg, 0.5626 mmol, 75.32 %, colorless oil).

^{1}-H NMR ($CDCl_3$): δ= 7.369-7.321 (3H, m), 7.189-7.077 (5H, m), 7.037-7.006 (2H, m), 6.963-6.936 (1H, m), 6.913-6.853 (2H, m), 5.071 (2H, s), 4.693-4.631(1H m), 3.951-3.912 (1H, m),

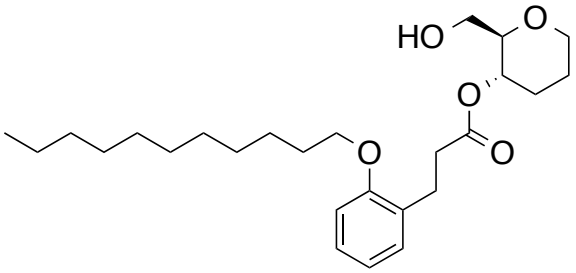
3.683-3.586 (2H, m), 3.357-3.256 (2H, m), 2.982-2.943 (2H, m), 2.607-2.567 (2H, m), 2.180-2.140 (1H, m), 1.769-1.560 (2H, m), 1.410-1.263 (1H, m), 0.871 (9H, s), 0.018-0.013 (6H, m).
^{13}C NMR ($CDCl_3$): δ= 172.18, 157.68, 156.91, 156.37, 139.33, 130.17, 129.91, 129.80, 129.05, 127.62, 123.48, 121.52, 120.85, 119.16, 117.89, 117.14, 111.61, 80.54, 69.33, 68.35, 67.59, 63.36, 34.37, 29.16, 26.20, 25.99, 24.95, 18.46, -5.27, -5.33.
HRMS (ESI-TOF [M+Na]$^+$) : Calcd. for $C_{34}H_{44}NaO_6Si^+$: 599.2799, Found 599.2816.
Anal. Calcd. for C, 69.47; H, 7.76; N, 0.00. Found C, 69.70; H, 7.53; N, 0.00.

Compound **B-16**



Compound **B-13** (113.3 mg, 0.2218 mmol) was dissolved in THF (1.2 mL) and hydrogen fluoride-pyridine complex (145.9 μL) in pyridine (0.324 mL) was added to the solution above. This reaction mixture was stirred at room temperature under Ar atmosphere for 12 hrs. After 12 hrs, the reaction mixture was diluted with CH_2Cl_2 (10 mL) and water (10 mL) was added to the solution. Organic layer was separated, washed with brine, dried over $MgSO_4$ and solvent was evaporated. The residue was purified by column chromatography (hexane : ethyl acetate = 4:1) to yield **B-16** (76.3 mg, 0.1924 mmol, 86.74 %, colorless oil).
^{1}H NMR ($CDCl_3$): δ= 5.389-5.291 (2H, m), 4.718-4.654 (1H, m), 3.994, 3.946 (1H, m), 3.675 (1H, dd, J= 12.0 Hz, 2.0 Hz), 3.551-3.507 (1H, m), 3.395 (1H, dt, J= 11.6 Hz, 2.8 Hz), 3.301-3.238 (1H, m), 2.349-2.262 (3H, m), 2.211-2.147 (1H, m), 2.065-1.960 (3H, m), 1.807-1.333 (5H, m), 1.293-1.246 (21H, m), 0.871 (3H, t, J= 6.8 Hz).
^{13}-C NMR ($CDCl_3$): δ= 173,37, 130.02, 129.71, 78.88, 68.10, 67.71, 62.34, 34.44, 31.92, 29.76, 29.67, 29.65, 29.52, 29.32, 29.31, 29.20, 29.13, 29.07, 27.22, 27.14, 25.08, 24.96, 22.67, 14.10.
HRMS (ESI-TOF [M+Na]$^+$): Calcd. for $C_{24}H_{44}NaO_4^+$: 419.3132, Found 419.3151.
Anal. Calcd. for C, 72.68; H, 11.18; N, 0.00. Found C, 72.39; H, 11.14; N, 0.00.

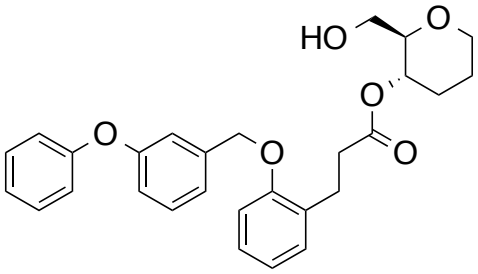
Compound **B-17**



Compound **B-14** (122.6 mg, 0.2234 mmol) was dissolved in THF (1.3 mL) and hydrogen fluoride-pyridine complex (157.9 μL) in pyridine (0.351 mL) was added to the solution above. This reaction mixture was stirred at room temperature under Ar atmosphere for 17 hrs. After 17 hrs, the reaction mixture was diluted with CH_2Cl_2 (8 mL) and water (10 mL) was added to the solution. Organic layer was separated, washed with brine, dried over $MgSO_4$ and solvent was evaporated. The residue was purified by column chromatography (hexane : ethyl acetate = 4:1) to yield **B-17** (83.7 mg, 0.1926 mmol, 86.2 %, colorless oil).

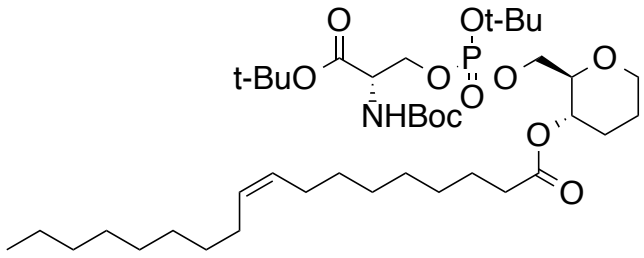
^{1}-H NMR ($CDCl_3$): δ= 7.192-7.113, (2H, m), 6.869-6.810 (2H, m), 4.713-4.649 (1H, m), 3.983-3.942 (3H, m), 3.573 (1H, dd, *J*= 12.4 Hz, 2.4 Hz), 3.440-3.344 (2H, m), 3.252-3.222 (1H, m), 2.947-2.910 (2H, m), 2.642-2.604 (2H, m), 2.260 (1H, brs), 2.166-2.126 (1H, m), 1.762-1.650 (4H, m), 1.516-1.413 (3H, m), 1.373-1.238 (14H, m), 0.900-0.866 (3H, m).
^{13}-C NMR ($CDCl_3$): δ= 172.99, 156.97, 129.96, 128.54, 127.66, 120.17, 111.05, 79.91, 68.14, 67.75, 67.71, 62.20, 34.27, 31.92, 29.64, 29.38, 29.36, 29.33, 29.17, 26.32, 26.18, 25.09, 22.69, 14.12.
HRMS (ESI-TOF $[M+Na]^+$): Calcd. for $C_{26}H_{42}NaO_5^+$: 457.2930, Found 457.2937.
Anal. Calcd. for C, 70.75; H, 9.60; N, 0.00. Found C, 70.59; H, 9.51; N, 0.00.

Compound **B-18**



Compound **B-15** (323.2 mg, 0.5603 mmol) was dissolved in THF (3.3 mL) and hydrogen fluoride-pyridine complex (345.5 μL) in pyridine (0.845 mL) was added to the solution above. This reaction mixture was stirred at room temperature under Ar atmosphere for 19 hrs. After 19 hrs, the reaction mixture was diluted with CH_2Cl_2 (10 mL) and water (10 mL) was added to the solution. Water layer was extracted with CH_2Cl_2 (10 mL×2), combined organic layer was washed with brine, dried over $MgSO_4$ and solvent was evaporated. The residue was purified by column chromatography (hexane : ethyl acetate = 2:1 to 1:1) to yield **B-18** (254.6 mg, 0.5505 mmol, 98.24 %, colorless oil).
^{1}-H NMR ($CDCl_3$): δ= 7.391-7.272 (3H, m), 7.226-7.125 (5H, m), 7.074-7.043 (2H, m), 6.997-6.971 (1H, m), 6.945-6.887 (2H, m), 5.082 (2H, s), 4.743-4.679 (1H, m), 3.985-3.946 (1H, m), 3.607-3.578 (1H, m), 3.465-3.334 (2H, m), 3.282-3.239 (1H, m), 3.007 (2H, t, *J*= 7.6 Hz), 2.675-2.616 (3H, m), 2.169-2.130 (1H, m), 1.805-1.637 (2H, m), 1.517-1.414(1H, m).
^{13}C NMR ($CDCl_3$): δ= 172.78, 157.72, 156.95, 156.40, 149.76, 139.35, 136.05, 130.25, 129.96, 129.85, 128.83, 127.76, 123.54, 121.58, 120.90, 119.19, 117.93, 117.17, 111.69, 79.95, 69.33, 68.25, 67.66, 62.15, 60.39, 34.28, 29.14, 26.32, 25.10, 14.24.
HRMS (ESI-TOF $[M+Na]^+$) : Calcd. for $C_{28}H_{30}NaO_6^+$: 485.1935, Found 485.1912.
Anal. Calcd. for C, 70.63; H, 6.39-; N, 0.00; (CH_2Cl_2×0.2). Found C, 70.61; H, 6.29; N, 0.00.

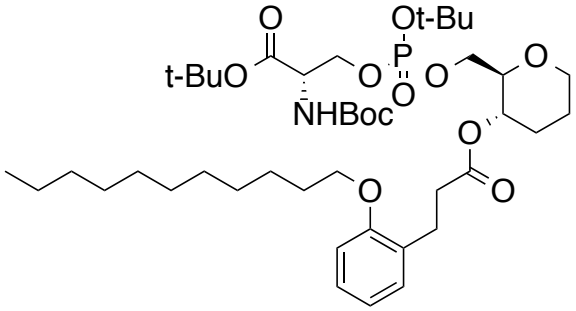
Compound **B-19**



A-3 (82.8 mg, 0.1834 mmol) and compound **B-16** (109.1 mg, 0.2751 mmol) was dissolved in CH_2Cl_2 and toluene and co-evaporated to remove containing water. The residue

was dissolved in CH_2Cl_2 (2 mL) and 1H-tetrazole (38.5 mg, 0.5501 mmol) in THF (2 mL) was added to the solution above. This reaction mixture was stirred at room temperature under Ar atmosphere for 2hrs 40min. After 2hrs 40min, *tert*-butylhydroperoxide 5 M in decane (0.0734 mg, 0.3668 mmol) was added to the reaction mixture and stirred at room temperature under Ar atmosphere for 1.5 hrs. After 1.5 hrs, water (10 mL) was added and the whole was extracted with CH_2Cl_2 (10 mL×2). Organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography (hexane : ethyl acetate = 4:1) to yield **B-19** (87.8 mg, 0.1131 mmol, 41.13 %, (62%; 2steps) colorless oil, starting material recovery: 50.3 mg, 46.10 %).
^{1}H NMR ($CDCl_3$): δ= 5.652-5.591 (1H, m), 5.359-5.289 (2H, m), 4.640-4.589 (1H, m), 4.353-4.308 (2H, m), 4.216-4.096 (1H, m), 4.091-3.941 (3H, m), 3.483-3.438 (1H, m), 3.387-3.331 (1H, m), 2.281-2.194 (3H, m), 2.049-1.911 (4H, m), 1.764-1.563 (4H, m), 1.504-1.395 (28H, m), 1.277-1.231 (20H, m), 0.873-0.838 (3H, m).
^{13}C NMR ($CDCl_3$): δ= 172.66, 172.65, 168.45, 168.41, 129.97, 129.71, 129.70, 83.45, 82.40, 79.73, 78.15, 67.92, 67.83, 67.62, 67.51, 66.79, 66.74, 66.52, 66.46, 34.34, 34.32, 31.87, 31.76, 31.49, 29.77, 29.76, 29.74, 29.73, 29.71, 29.68, 29.62, 29.58, 29.49, 29.30, 29.28, 29.22, 29.18, 29.14, 29.09, 28.32, 27.96, 27.93, 27.20, 27.15.
^{31}P-NMR ($CDCl_3$): δ= -5.53, -5.72.
HRMS (ESI-TOF $[M+Na]^+$): Calcd. for $C_{40}H_{74}NNaO_{11}P^+$: 798.4892, Found 798.4903.
Anal. Calcd. for C, 61.91; H, 9.61; N, 1.81. Found C, 61.65; H, 9.78; N, 1.83.

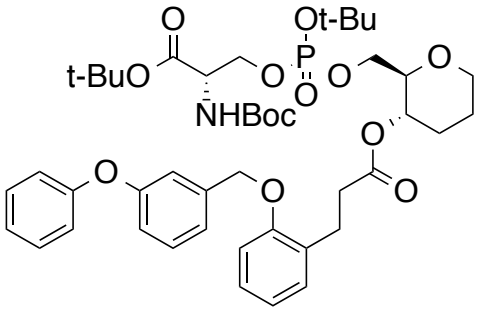
Compound **B-20**



A-3 (52.7 mg, 0.1167 mmol) and compound **B-17** (76.1 mg, 0.1751 mmol) was dissolved in CH_2Cl_2 (1.3 mL) and toluene (0.3 mL) and co-evaporated to remove containing water. The residue was dissolved in CH_2Cl_2 (1.3 mL) and *1H*-tetrazole (24.5 mg, 0.3501 mmol) in THF (1.3 mL) was added to the solution above. This reaction mixture was stirred at room temperature under Ar atmosphere for 15 hrs. After 15 hrs, *tert*-butylhydroperoxide 5 M in decane (0.047 mg, 0.2334 mmol) was added to the reaction mixture and stirred at room temperature under Ar atmosphere for 3 hrs. After 3 hrs, water (5 mL) was added and the whole was extracted with CH_2Cl_2 (7 mL×2). Organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography (hexane : ethyl acetate = 4:1 to 2:1) to yield **B-20** (26.7 mg, 0.0328 mmol, 28.1 % (2 steps), colorless oil, starting material recovery: 47.8 mg, 62.8 %).
^{1}H NMR ($CDCl_3$): δ= 7.110-7.044 (2H, m), 6.793-6.735 (2H, m), 5.614-5.556 (1H, m), 4.600-4.507 (1H, m), 4.302-4.226 (2H, m), 4.173-4.125 (1H, m), 4.008-3.856 (5H, m), 3.690-3.657 (1H, m), 3.412-3.367 (1H, m), 3.323-3.265 (1H, m), 2.887-2.789 (2H, m), 2.563-2.518 (2H, m), 2.152-2.105 (1H, m), 1.797-1.530 (5H, m), 1.415-1.374 (27H, m), 1.301-1.199 (15H, m), 0.828-0.793 (3H, m).
^{13}C NMR ($CDCl_3$): δ= 172.29, 168.50, 168.45, 156.93, 155.43, 129.95, 129.92, 128.68, 128.62, 127.58, 127.55, 120.16, 111.03, 83.77, 83.70, 82.45, 82.41, 79.74, 78.20, 78.16, 78.13, 68.00, 67.96, 67.90, 67.75, 67.59, 67.46, 66.80, 66.74, 66.51, 66.45, 54.47, 34.18, 31.91, 29.79, 29.78,

29.75, 29.73, 29.64, 29.63, 29.38, 29.34, 29.32, 29.14, 29.08, 28.35, 27.97, 27.95, 26.14, 26.11, 25.61, 24.81, 24.75, 22.67, 14.10.
^{31}P-NMR ($CDCl_3$): δ= -5.52, -5.77.
HRMS (ESI-TOF [M+Na]$^+$): Calcd. for $C_{42}H_{72}NNaO_{12}P^+$: 836.4690, Found 836.4692.
Anal. Calcd. for C, 61.48; H, 8.85; N, 1.70; (CH_2Cl_2X0.1). Found C, 61.41; H, 8.47; N, 1.76.

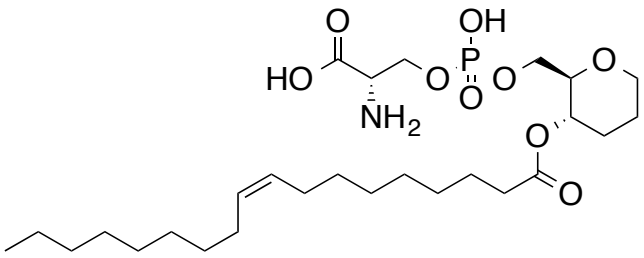
Compound **B-21**



Compound **A-3** (89.2 mg, 0.1976 mmol) and **B-18** (137.1 mg, 0.1604 mmol) was dissolved in CH_2Cl_2 (1 mL) and toluene (0.1 mL) and co-evaporated. The residue was dissolved in CH_2Cl_2 (1 mL) and *1H*-tetrazole(16.0 mg, 0.2291 mmol) in THF (1 mL) was added to the solution above. The whole was stirred at room temperature under Ar atmosphere for 19 hrs. After 19 hrs, the reaction mixture was diluted with CH_2Cl_2 (8 mL) and quenched by saturated aqueous $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (8 mL×2). Combined organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography (hexane : ethyl acetate : NEt_3 = hexane: ethyl acetate) to yield trivalent phosphodiester compound (65.8 mg, 0.0797 mmol, 40.33 %, colorless oil).

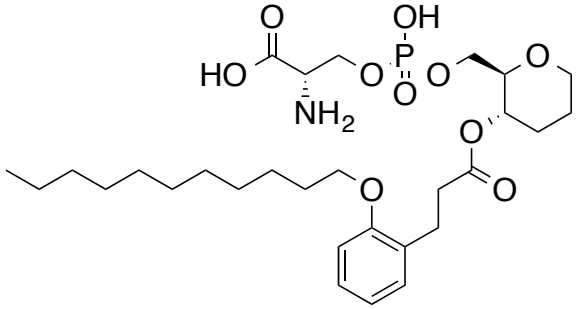
The trivalent phosphodiester compound (65.7 mg, 0.0795 mmol) was dissolved in CH_2Cl_2 (1mL) *tert*-butylhydroperoxide in decane (0.0318 mg). The reaction mixture was stirred at room temperature under Ar atmosphere for 1.5 hr. After 1.5 hr, solvent was removed under vacuum and the residue was purified by column chromatography (hexane : acetone = 4:1) to yield **B-21** (60.8 mg, 0.0722 mmol, 90.84 %, colorless oil (45.02 % (2 steps)).
^{1}H NMR ($CDCl_3$): δ= 7.262-7.216 (3H, m), 7.084-6.994 (5H, m), 6.939-6.909 (2H, m), 6.868-6.842 (1H, m), 6.817-6.767 (2H, m), 5.491 (1H, brs), 4.986 (2H, s), 4.581-4.493 (1H, m), 4.249-4.232 (2H, m), 4.157-4.107 (1H, m), 3.983-3.831 (3H, m), 3.379-3.333 (1H, m), 3.292-3.216 (1H, m), 2.908-2.859 (2H, m), 2.550-2.504 (2H, m), 2.102 (1H, m), 1.705-496 (2H, m), 1.395-1.173 (28H, m).
^{13}C NMR ($CDCl_3$): δ= 172.00, 171.97, 168.43, 168.40, 157.73, 157.05, 156.46, 139.43, 139.40, 130.11, 130.07, 129.83, 129.71, 129.14, 129.09, 127.57, 127.54, 123.39, 121.56, 120.92, 119.09, 117.91, 117.90, 117.24, 111.90, 83.55, 82.35, 82.41, 78.22, 78.20, 28.15, 78.13, 69.57, 68.12, 68.04, 67.47, 67.34, 66.82, 66.76, 66.56, 66.50, 34.25, 29.77, 29.72, 29.08, 29.02, 28.32, 27.97, 27.95, 26.05, 26.04, 24.78, 24.73.
^{31}P-NMR ($CDCl_3$): δ= -5.57, -5.79.
HRMS (ESI-TOF [M+Na]$^+$): Calcd. for $C_{44}H_{60}NNaO_{13}P^+$: 864.3700, Found 864.3701.
Anal. Calcd. for C, 60.58; H, 7.27; N, 1.61. Found C, 60.18; H, 6.89; N, 1.59.

Compound **B-22**



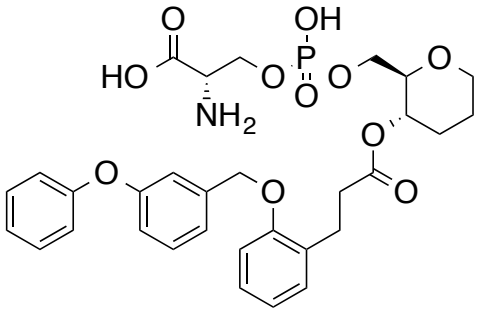
Compoud **19** (82.5 mg, 0.1063 mmol) was dissolved in trifluoroacetic acid (2 mL) at 0 ℃, stirred at 0 ℃ for 10 min and stirred at room temperature for 2 hrs. After 2 hrs, the reaction mixture was evaporated to remove solvent and the residue was purified by column chromatography ($CHCl_3$: MeOH : AcOH = 7:1:1 to 6:1:3) to yield **B-22** (57.2 mg, 0.1015 mmol, 95.47 % (TFA salt), white solid).
^{1}H NMR ($CDCl_3$): δ= 5.547-5.276 (1H, m), 5.118-5.056 (1H, m), 4.904 (1H, brs), 4.630-4.530 (3H, m), 4.168-4.076 (3H, m), 3.658-3.529 (2H, m), 2.327-2.333 (2H, m), 2.238-2.215 (1H, m), 2.043-1.974 (2H, m), 1.809-1.592 (7H, m), 1.266 (20H, brs), 0.892-0.858 (3H, m).
^{31}P-NMR ($CDCl_3$): δ= -1.71.
HRMS (ESI-TOF: $[M-H]^-$): Calcd. for $C_{27}H_{49}NO_9P^-$: 562.3150, Found 562.3152.
Anal. Calcd. for C, 50.07; H, 7.68; N, 2.01; ($CF_3CO_2H\times1$, $H_2O\times1$). Found C, 49.69; H, 7.37; N, 2.07.

Compound **B-23**



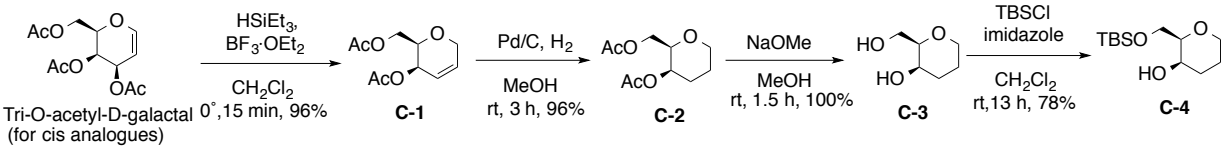
Compoud **B-20** (20.6 mg, 0.0253 mmol) was dissolved in trifluoroacetic acid (2 mL) at 0 ℃, stirred at 0 ℃ for 10 min and stirred at room temperature for 2 hrs. After 2 hrs, the reaction mixture was evaporated to remove solvent and the residue was purified by column chromatography ($CHCl_3$:MeOH: AcOH = 7:1:1 to 6:1:4) to yield **B-23** (16 mg, 0.0266 mmol, 105 % (TFA salt), white solid).
^{1}H NMR ($CDCl_3$): δ= 7.098-7.062 (1H, m), 6.944-6.926 (1H, m), 6.774-6.737 (2H, m), 4.744 (1H, brs), 4.495-4.395 (3H, m), 3.958-3.815 (5H, m), 3.451-3.366 (2H, m), 2.811-2.798 (2H, m), 2.679-2.590 (2H, m), 1.980-1.956 (1H, m), 1.706-1.637 (4H, m), 1.397-1.106 (14H, m), 0.776-0.742 (3H, m).
^{31}P-NMR ($CDCl_3$): δ= -1.69.
HRMS (ESI-TOF: $[M-H]^-$): Calcd. for $C_{29}H_{47}NO_{10}P^-$: 600.2943, Found 600.2917.

Compound **B-24**

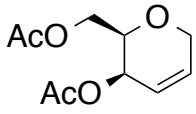


Compound **B-21** (62.5 mg, 0.0742 mmol) was dissolved in trifluoroacetic acid (2 mL) at 0 ℃ and stirred at room temperature for 2.5 hrs. After 2.5 hrs, solvent was removed under vacuum and the residue was purified by column chromatography ($CHCl_3$: MeOH : AcOH = 6:1:2 to 5:1:4) to yield **B-24** (50.9 mg, 0.0808 mmol, 108.96 % (TFA salt), white solid).
^{1}H NMR ($CDCl_3$): δ= 7.386-7.343 (3H, m), 7.239-7.094 (5H, m), 7.041-7.022 (2H, m), 6.988-6.908 (3H, m), 5.115 (2H, s), 4.859 (1H, m), 4.628 (2H, m), 4.497-4.444 (1H, m), 4.094-4.025 (3H, m), 3.566-3.549 (1H, m), 3.462 (1H, m), 2.995-2.960 (2H, m), 2.789-2.690 (2H, m), 2.064-2.041(1H, m), 1.727 (2H, m), 1.515-1.417 (1H, m).
^{31}P-NMR ($CDCl_3$): δ= -1.86.
HRMS (ESI-TOF [M-H]$^-$): Calcd. for $C_{31}H_{35}NO_{11}P^-$: 628.1953, Found 628.1959.
Anal. Calcd. for C, 53.30; H, 5.02; N, 1.88. Found C, 53.37; H, 5.11; N, 1.79.

5. Synthesis of modified glycerol backbone (cis)



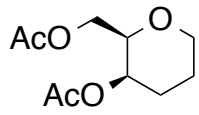
Compound **C-1**



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. BF_3·OEt_2 (3.2776 g, 23.0929 mmol) was added dropwise to the solution above. The reaction mixture was stirred at room temperature under Ar for 15 min. Saturated aqueous $NaHCO_3$ solution (50 mL) was added to the reaction mixture and the whole was extracted with CH_2Cl_2 (20 mL×3). 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 **C-1** (3.9502 mg, 18.3499 mmol, 95.82 %, colorless oil).
^{1}H NMR ($CDCl_3$): δ= 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 ($CDCl_3$): δ= 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.

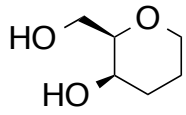
Anal. Calcd. for C, 56.07; H, 6.59; N, 0.00. Found C, 55.90; H, 6.40; N, 0.00.

Compound **C-2**



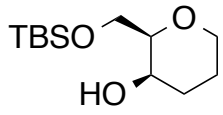
Compound **C-1** (555.6 mg, 2.5936 mol) was dissolved in MeOH (7 mL) and 10 % Pd/C (56 mg) in MeOH (3 mL) was added to the solution above. The reaction mixture was placed under H_2 atmosphere and stirred at room temperature for 3 hrs. After 3 hrs, the reaction mixture was filtered on celite and the filtrate was evaporated to yield **C-2** (539.6 mg, 2.4955 mmol, 96.2 %, colorless oil).
^{1}H NMR ($CDCl_3$): δ= 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 ($CDCl_3$): δ= 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.

Compound **C-3**



Compound **C-2** (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. The reaction mixture was stirred at room temperature under Ar for 1.5 hrs. After 1.5 hrs, 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 **C-3** (397.6 mg, 3.0085 mmol, >100 %, yellow oil).
^{1}H NMR ($CDCl_3$): δ= 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 ($CDCl_3$): δ= 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.

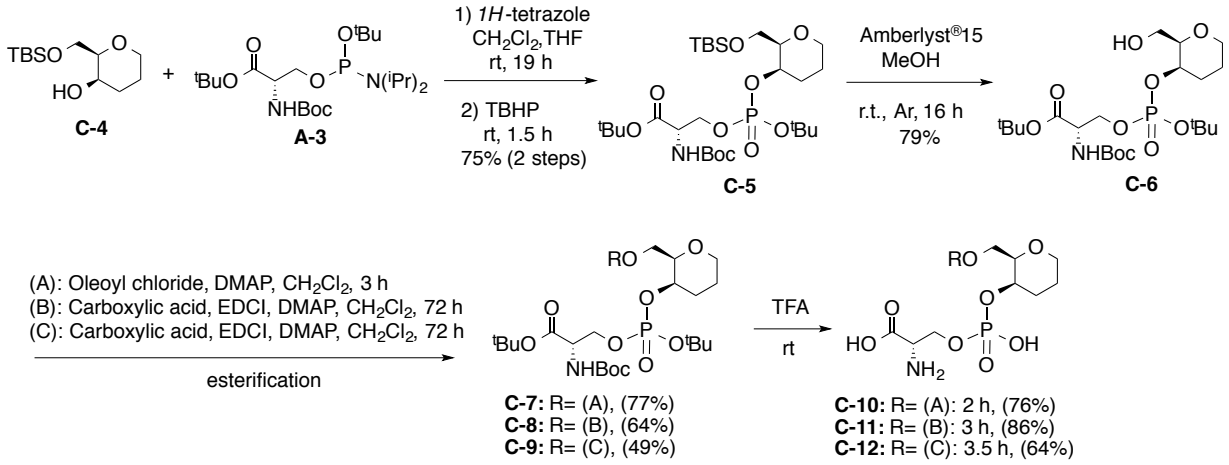
Compound **C-4**



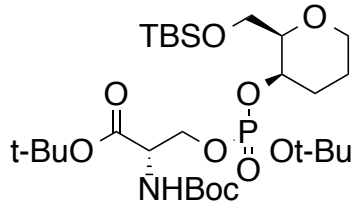
Compound **C-3** (32.5 mg, 0.2459 mmol) was dissolved in CH_2Cl_2 (0.7 mL) and imidazole (36.8 mg, 0.5410 mmol) was added to the solution. This was cooled to 0 ℃ and tert-butyldimethylsilyl chloride (44.5 mg, 0.2951 mmol) was added. The reaction mixture was stirred at room temperature under Ar for 13 hrs. After, 13 hrs, H_2O (5 mL) was added to the reaction mixture and the whole was extracted CH_2Cl_2 (5 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) to yield **C-4** (47.0 mg, 0.1907 mmol, 77.6 %, colorless oil).

^{1}-H NMR ($CDCl_3$): δ= 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 ($CDCl_3$): δ= 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 $C_{12}H_{26}NaO_3^+$: 269.1543, Found 269.1554.
Anal. Calcd. for C, 58.49; H, 10.63; N, 0.00. Found C, 58.19; H, 10.39; N, 0.00.

6. Synthesis of cis-1˚-acyl-2˚-phosphoserine



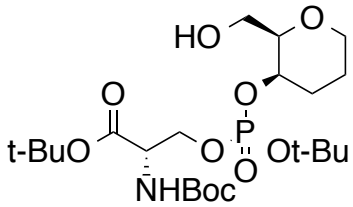
Compound **C-5**



A-3 (439.2 mg, 0.9726 mmol) and compound **C-4** (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.

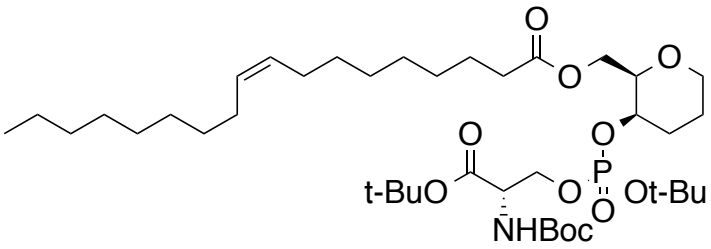
The residue was dissolved in dry CH_2Cl_2 (6.5 mL) and *1H*-tetrazole (272.5 mg, 3.8904 mmol) in THF (6.5 mL) was added to the solution. This reaction mixture was stirred at room temperature under Ar for 19 hrs. After 17 hrs, *tert*-butylhydroperoxide (0.3890 mg, 1.9452 mmol) was added to the reaction mixture and stirred at room temperature under Ar for 1.5 hrs. After 1.5 hrs, the reaction mixture was quenched by water (15 mL) and the whole was 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 (hexane : ethyl acetate = 4:1 to 2:1) to yield **C-5** (456.6 mg, 0.7296 mmol, 50.0 %, colorless oil, (75.02 % (2 steps)))
^{1}H NMR ($CDCl_3$): δ= 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 ($CDCl_3$): δ= 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 ($CDCl_3$): δ= -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, 53.74; H, 9.02; N, 2.24. Found C, 53.61; H, 9.08; N, 2.20.

Compound **C-6**



Compound **C-5** (428.2 mg, 0.6842 mmol) was dissolved in MeOH (17 mL) and cooled to 0 ℃. Amberlyst®15 (2.0021 g) was added to the solution above, and stirred at 0 ℃ under Ar for 10 min. After 10 min, the reaction mixture was stirred at room temperature under Ar for 16 hrs. After 16hrs, reaction mixture was filtered on celite and the filtrate was evaporated. The residue was purified by column chromatography (ethyl acetate) to yield **C-6** (275.2 mg, 0.5380 mmol, 78.6 %, colorless oil).
^{1}H NMR ($CDCl_3$): δ= 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 ($CDCl_3$): δ= 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 ($CDCl_3$): δ= -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, 51.65; H, 8.28; N, 2.74. Found C, 51.58; H, 7.98; N, 2.57.

Compound **C-7**



Compound **C-6** (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 ℃ 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 hrs. After 3 hrs, methanol (2 mL) was added and stirred at room temperature for 16 hrs. After 16 hrs, solvent was removed under vacuum and the residue was purified by column chromatography (hexane : ethyl acetate = 4:1 to 2:1) to yield **C-7** (115.6 mg, 0.1490 mmol, 76.7 %, colorless oil).

^{1}H NMR ($CDCl_3$): δ= 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).

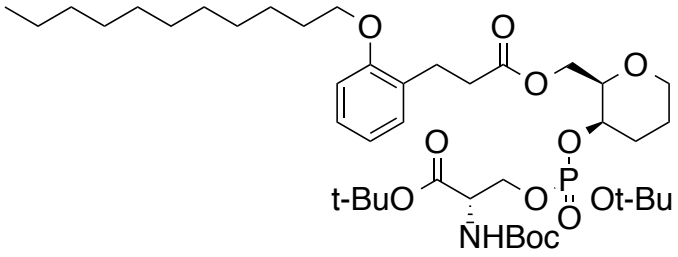
^{13}C NMR ($CDCl_3$): δ= 173.58, 168.35, 155.22, 129.97, 129.95, 129.72, 83.88, 83.81, 82.64, 82.61, 79.88, 79.80, 76.36, 76.29, 71.86, 71.80, 71.59, 71.54, 67.83, 67.75, 64.37, 64.21, 54.43, 54.35, 34.09, 31.88, 29.85, 29.81, 29.77, 29.74, 29.69, 29.68, 29.49, 29.33, 29.32, 29.30, 29.17, 29.15, 29.11, 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 ($CDCl_3$): δ= -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, 61.43; H, 9.46; N, 1.79; (CH_2Cl_2X0.1). Found C, 61.46; H, 9.36; N, 1.77.

Compound **C-8**

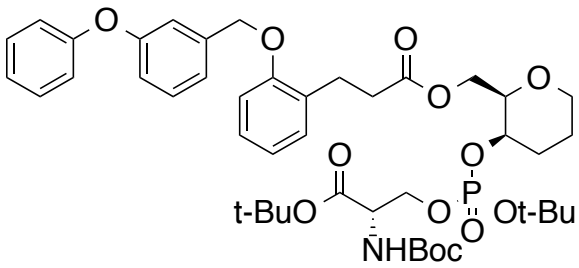


Compound **C-6** (117.8 mg, 0.2303 mmol) and carboxylic acid derivative (**B**) (95.9 mg, 0.2994 mmol) were dissolved in CH_2Cl_2 (4.3 mL) EDCI (57.4 mg, 0.2994 mmol) and *N,N*-dimethylaminopyridine (7.4 mg, 0.0606 mmol) were added to the reaction mixture. This was stirred at room temperature under Ar 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 hrs. 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 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 = 2:1 to 1:1) to yield **C-8** (120.1 mg, 0.1475 mmol, 64.1 %, colorless oil).

^{1}H NMR ($CDCl_3$): δ= 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

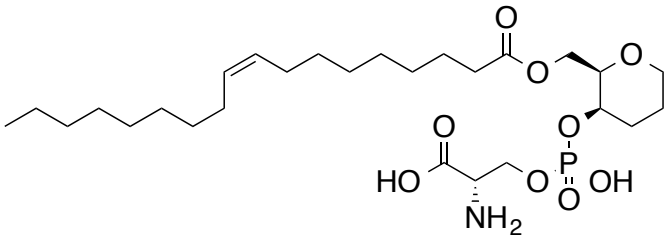
(2H, t, *J*= 6.4 Hz), 3.556-3.520 (1H, m), 3.439 (1H, dt, *J*= 12.0 Hz, 2.0 Hz), 2.942-2.897 (2H, m), 2.663-2.610 (2H, m), 2.264-2.170 (1H, m), 2.047-1.907 (1H, m), 1.802-1.732 (2H, m), 1.688-1.603 (1H, m), 1.477-1.417 (30H, m), 1.378-1.250 (14H, m), 0.862 (3H, t, *J*= 6.4 Hz).
^{13}-C NMR ($CDCl_3$): δ= 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 ($CDCl_3$): δ= -5.29, -5.85.
HRMS (ESI-TOF $[M+Na]^+$): Calcd. for $C_{42}H_{72}NNaO_{12}P^+$: 836.4648, Found 836.4685.
Anal. Calcd. for C, 61.45; H, 8.88; N, 1.71; (H_2OX0.4). Found C, 61.09; H, 8.66; N, 1.73.

Compound **C-9**



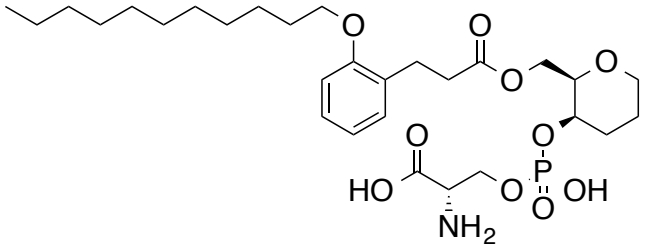
Compound **C-6** (98.1 mg, 0.1918 mmol) and carboxylic acid derivative (**C**) (86.9 mg, 0.2493 mmol) were dissolved in CH_2Cl_2 (43.6 mL) EDCI (47.8 mg, 0.2493 mmol) and *N*,*N*-dimethylaminopyridine (3.0 mg, 0.0249 mmol) were added to the reaction mixture. This was stirred at room temperature under 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 hrs. 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 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 = 2:1 to 1:1) to yield **C-9** (79.8 mg, 0.0948 mmol, 49.4 %, colorless oil).
^{1}H-NMR ($CDCl_3$): δ= 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), 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 ($CDCl_3$): δ= 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 ($CDCl_3$): δ= -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, 62.77; H, 7.18; N, 1.66. Found C, 59.53; H, 7.38; N, 1.71.

Compound **C-10**



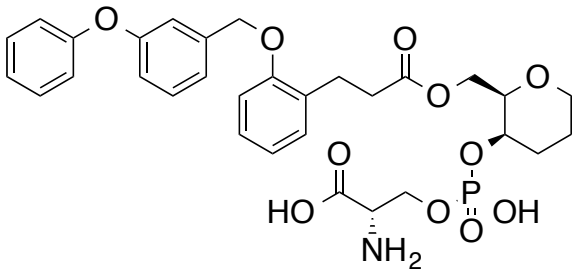
Compound **C-7** (113.6 mg, 0.1464 mmol) was dissolved in trifluoroacetic acid (2 mL) at 0 ℃ and stirred at room temperature for 2 hrs. After 2 hrs, solvent was removed under vacuum and the residue was purified by column chromatography ($CHCl_3$: MeOH : AcOH = 8:1:1 to 6:1:3) to yield **C-10** (62.4 mg, 0.1107 mmol, 75.62 %, white solid).
^{1}H NMR ($CDCl_3$): δ= 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 ($CDCl_3$): δ= -2.87.
HRMS (ESI-TOF: $[M-H]^-$): Calcd. for $C_{27}H_{49}NO_9P^-$: 562.3150, Found 562.3182.
Anal. Calcd. for C, 51.40; H, 7.59; N, 2.07; (CF_3COOH X 1). Found C, 51.00; H, 7.47; N, 2.02.

Compound **C-11**



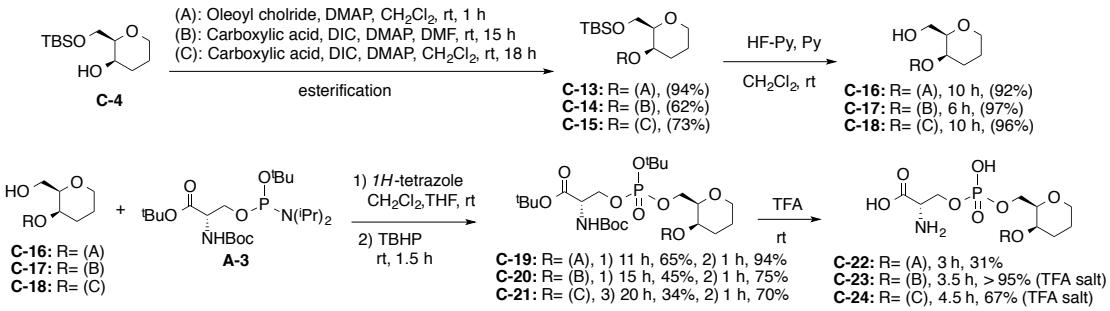
Compound **C-8** (126.7 mg, 0.1557 mmol) was dissolved in trifluoroacetic acid (2 mL) at 0 ℃ and stirred at room temperature for 3 hrs. After 3 hrs, solvent was removed under vacuum and the residue was purified by column chromatography ($CHCl_3$: MeOH : AcOH = 8:1:1 to 6:1:3) to yield **C-11** (80.38 mg, 0.1336 mmol, 85.80 %, white solid).
^{1}H NMR ($CDCl_3$): δ= 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 ($CDCl_3$): δ= -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, 53.38; H, 7.15; N, 2.06; (CF_3COOH×0.7). Found C, 53.75; H, 7.30; N, 2.08.

Compound **C-12**

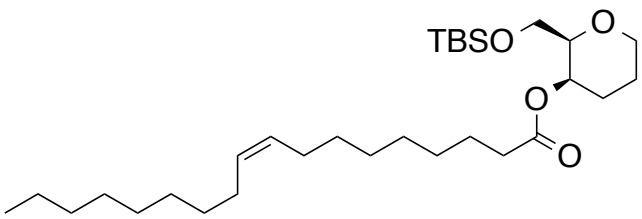


Compound **C-9** (74.0 mg, 0.0879 mmol) was dissolved in trifluoroacetic acid (2 mL) at 0 ℃ and stirred at room temperature for 3.5 hrs. After 3.5 hrs, solvent was removed under vacuum and the residue was purified by column chromatography ($CHCl_3$: MeOH : AcOH = 8:1:1 to 6:1:3) to yield **C-12** (35.25 mg, 0.0560 mmol, 63.70 %, white solid).
^{1}H NMR ($CDCl_3$): δ= 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 ($CDCl_3$): δ= 0.97.
HRMS (ESI-TOF: [M-H]$^-$): Calcd. for $C_{31}H_{35}NO_{11}P^-$: 628.1953, Found 628.1915.
Anal. Calcd. for C, 52.34; H, 4.89; N, 1.83; (CF_3CO_2H×1.2). Found C, 52.28; H, 4.99; N, 1.71.

7. Synthesis of cis-2˚-acyl-1˚-phosphoserine



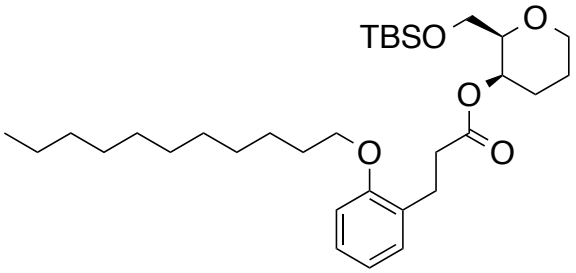
Compound **C-13**



Compound **C-4** (200.0 mg, 0.8116 mmol) was dissolved in CH_2Cl_2 (3 mL), *N*,*N*-dimethylaminopyridine (297.5 mg, 2.4349 mmol) was added to the solution and the mixture was cooled to 0 ℃. Oleoyl chloride (317.5 mg, 1.0551 mmol) was added to the solution above and stirred at 0 ℃ to room temperature under Ar for 1 hr. After 1 hr, the reaction mixture was evaporated under vacuum to remove solvent and the residue was purified by column chromatography (hexane : ethyl acetate = 16:1) to yield **C-13** (389.5 mg, 0.7624 mmol, 93.9 %, colorless oil).

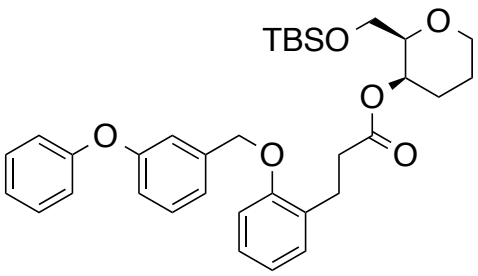
^{1}H NMR ($CDCl_3$): δ= 5.375-5.291 (2H, m), 4.978 (1H, brs), 4.029-3.990 (1H, m), 3.634-3.459 (4H, m), 2.353-2.314 (2H, m), 2.094-1.913 (5H, m), 1.890-1.804 (1H, m), 1.707-1.593 (3H, m), 1.421-1.248 (21H, m), 0.872-0.851 (12H, m), 0.021-0.012 (6H, m).
^{13}C NMR ($CDCl_3$): δ= 173.21, 129.98, 129.73, 78.67, 68.08, 66.46, 62.09, 34.56, 31.92, 29.76, 29.70, 29.68, 29.66, 29.64, 29.60, 29.51, 29.35, 29.33, 29.31, 29.27, 29.19, 29.16, 29.14, 29.11, 27.49, 27.21, 27.17, 25.82, 25.15, 22.66, 20.93, 18.28, 14.09.
HRMS (ESI-TOF [M+Na]$^+$) : Calcd. for $C_{30}H_{58}NaO_4Si^+$: 533.3997, Found 533.3997.

Compound **C-14**



Carboxylic acid derivative (**B**) (104.4 mg, 0.3258 mmol) was dissolved in CH_2Cl_2 (1 mL), diisopropylcarbodiimide (61.7mg, 0.4887 mmol) and *N,N*-dimethylaminopyridine (4 mg, 0.0326 mmol) was added to the solution above. Compound **C-4** (80.3 mg, 0.3258 mmol) was added to the reaction mixture and the whole was stirred at room temperature under Ar atmosphere for 15 hrs. After 15 hrs, the reaction mixture was diluted with CH_2Cl_2 (10 mL) and quenched by water (10 mL). Water layer 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 (hexane : ethyl acetate = 10:1 to 5:1) to yield **C-14** (111.4 mg, 0.2030 mmol, 62.31 %, colorless oil).
^{1}H NMR ($CDCl_3$): δ=7.182-7.137 (2H, m), 6.865-6.804 (2H, m), 4.993 (1H, brs), 4.013-3.941 (3H, m), 3.623-3.543 (2H, m), 3.515-3.449 (2H, m), 2.985-2.946 (2H, m), 2.694-2.654 (2H, m), 2.060-2.006 (1H, m), 1.841-1.727 (3H, m), 1.702-1.602 (1H, m), 1.511-1.438 (2H, m), 1.373-1.277 (15H, m), 0.921-0.836 (12H, m), 0.025-0.012 (6H, m).
^{13}C NMR ($CDCl_3$): δ= 172.81, 156.99, 129.91, 128.88, 127.48, 120.14, 110.97, 78.64, 68.06, 67.76, 66.58, 62.10, 34.31, 31.93, 29.66, 29.64, 29.61, 29.42, 29.37, 29.36, 27.50, 26.28, 26.16, 25.84, 22.69, 20.87, 18.22, 14.12.
HRMS (ESI-TOF [M+Na]$^+$) : Calcd. for $C_{32}H_{56}NaO_5Si^+$: 571.3789, Found 571.3745.
Anal. Calcd. for C, 70.02; H, 10.28; N 0.00. Found C, 69.74; H, 9.98; N, 0.00.

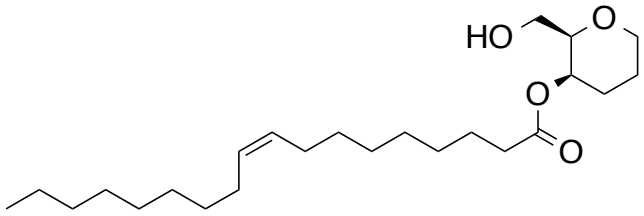
Compound **C-15**



Carboxylic acid derivative (**C**) (50.4 mg, 0.2047 mmol) was dissolved in CH_2Cl_2 (1 mL), diisopropylcarbodiimide (33.6 mg, 0.2661 mmol) and *N,N*-dimethylaminopyridine (2.5 mg, 0.0205 mmol) was added to the solution above. Compound **C-4** (71.3 mg, 0.2047 mmol)

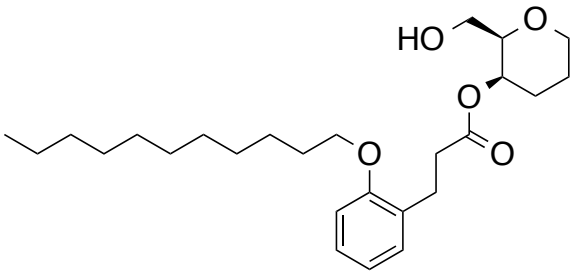
was added to the reaction mixture and the whole was stirred at room temperature under Ar atmosphere for 18 hrs. After 18 hrs, diisopropylcarbodiimide (0.3 eq) was added to the reaction mixture and stirred at room temperature under Ar atmosphere for 5 hrs. After 5 hrs, the reaction mixture was diluted with CH_2Cl_2 (10 mL) and quenched by water (10 mL). Water layer 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 (hexane : ethyl acetate = 9:1 to 5:1) to yield **C-15** (85.9 mg, 0.1489 mmol, 72.75 %, colorless oil).
^{1}H NMR ($CDCl_3$): δ= 7.369-7.323 (3H, m), 7.208-7.099 (4H, m), 7.077-7.017 (3H, m), 6.969-6.851 (3H, m), 5.072 (2H, s), 4.990 (1H, brs), 4.006-4.967 (1H, m), 3.617-3.442 (4H, m), 3.009 (2H, t, *J*= 7.6 Hz), 2.746-2.618 (2H, m), 2.033-1.988 (1H, m), 1.801-1.592 (2H, m), 1.349-1.275 (1H, m), 0.866 (9H, s), 0.000 (3H, s), -0.019 (3H, s).
^{13}C NMR ($CDCl_3$): δ= 172.72, 157.64, 156.96, 156.41, 139.36, 130.14, 129.97, 129.80, 129.14, 127.56, 123.46, 121.57, 120.83, 119.17, 117.91, 117.13, 111.59, 78.65, 69.35, 68.06, 66.69, 62.12, 34.25, 27.50, 26.21, 25.86, 20.85, 18.23, -5.46, -5.55.
HRMS (ESI-TOF $[M+Na]^+$) : Calcd. for $C_{34}H_{44}NaO_6Si^+$: 599.2799, Found 599.2792.
Anal. Calcd. for C, 70.80; H, 7.69; N 0.00. Found C, 70.71; H,7.62; N, 0.00.

Compound **C-16**



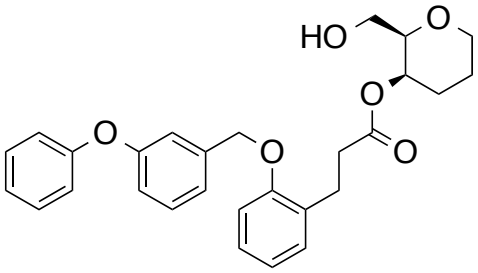
Compound **C-13** (389.3 mg, 0.7620 mmol) was dissolved in THF (4.5 mL) and hydrogen fluoride-pyridine complex (451.2 μL) in pyridine (1 mL) was added to the solution above. This reaction mixture was stirred at room temperature under Ar atmosphere for 10 hrs. After 10 hrs, the reaction mixture was diluted with CH_2Cl_2 (10 mL) and water (10 mL) was added to the solution. Organic layer was separated and extracted with CH_2Cl_2 (10 mL×1). Combined organic layer was washed with brine, dried over $MgSO_4$ and solvent was evaporated. The residue was purified by column chromatography (hexane : ethyl acetate = 4:1) to yield **C-16** (277.3 mg, 0.6992 mmol, 91.76 %, colorless oil).
^{1}H NMR ($CDCl_3$): δ= 5.401-5.290 (2H, m), 4.983 (1H, m), 4.107-4,032 (1H, m), 3.646-3.589 (1H, m), 3.568-3.484 (2H, m), 3.447-3.373 (1H, m), 2.423-2.354 (3H, m), 2,071-1.894 (5H, m), 1.793-1.606 (3H, m), 1.489-1.438 (1H, m), 1.305-1.254 (20H, m), 0.903-0.859 (3H, m).
^{13}C NMR ($CDCl_3$): δ= 174.27, 130.01, 129.71, 78.58, 68.11, 66.87, 62.09, 34.43, 31.89, 29.76, 29.67, 29.51, 29.31, 29.30, 29.14, 29.09, 28.97, 27.90, 27.21, 27.15, 25.06, 22.66, 20.89, 14.08.
HRMS (ESI-TOF $[M+Na]^+$) : Calcd. for $C_{24}H_{44}NaO_4^+$: 419.3132, Found 419.3153.
Anal. Calcd. for C, 72.68; H, 11.18; N, 0.00. Found C, 72.42; H, 10.94; N, 0.00.

Compound **C-17**



Compound **C-14** (197.2 mg, 0.1771 mmol) was dissolved in THF (1.3 mL) and hydrogen fluoride-pyridine complex (109.2 μL) in pyridine (0.267 mL) was added to the solution above. This reaction mixture was stirred at room temperature under Ar atmosphere for 6 hrs. After 6 hrs, the reaction mixture was diluted with CH_2Cl_2 (10 mL) and water (10 mL) was added to the solution. Water layer was extracted with CH_2Cl_2 (10 mL×2), combined organic layer was washed with brine, dried over $MgSO_4$ and solvent was evaporated. The residue was purified by column chromatography (hexane : ethyl acetate = 2:1) to yield **C-17** (75.0 mg, 0.1726 mmol, 97.44 %, colorless oil).
^{1}H NMR ($CDCl_3$): δ= 7.188-7.127 (2H, m), 6.864-6.808 (2H, m), 4.953-4.946 (1H, m), 4.038-3.998 (1H, m), 3.956 (2H, t, *J*= 6.4 Hz), 3.558-3.470 (3H, m), 3.341-3.266 (1H, m), 2.962 (2H, t, *J*= 7.6 Hz), 2.703 (2H, t, *J*= 7.4 Hz), 2.490-2.475 (1H, m), 1.960-1.655 (5H, m), 1.506-1.269 (17H, m), 0.881 (3H, t, *J*= 6.8 Hz).
^{13}C NMR ($CDCl_3$): δ= 173.92, 156.97, 129.93, 128.55, 127.64, 120.14, 111.02, 78.50, 68.08, 67.76, 66.95, 61.94, 34.22, 31.92, 29.64, 29.61, 29.39, 29.36, 29.34, 27.84, 26.34, 26.17, 22.69, 20.85, 14.13.
HRMS (ESI-TOF $[M+Na]^+$) : Calcd. for $C_{26}H_{42}NaO_5^+$: 457.2924, Found 457.2920.
Anal. Calcd. for C, 71.85; H, 9.74; N, 0.00. Found C, 71.57; H, 9.68; N, 0.00.

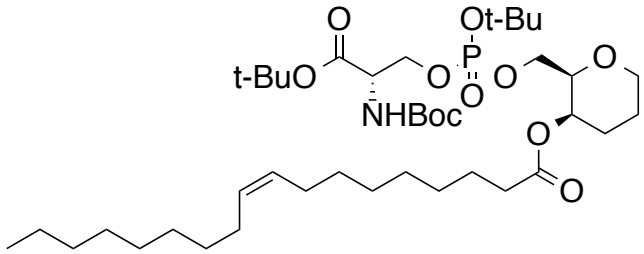
Compound **C-18**



Compound **C-15** (79.0 mg, 0.1327 mmol) was dissolved in THF (1 mL) and hydrogen fluoride-pyridine complex (81.1 μL) in pyridine (0.2 mL) was added to the solution above. This reaction mixture was stirred at room temperature under Ar atmosphere for 10 hrs. After 10 hrs, the reaction mixture was diluted with CH_2Cl_2 (6 mL) and water (10 mL) was added to the solution. Water layer was extracted with CH_2Cl_2 (6 mL×2), combined organic layer was washed with brine, dried over $MgSO_4$ and solvent was evaporated. The residue was purified by column chromatography (hexane : ethyl acetate = 1:1) to yield **C-18** (61.0 mg, 0.1319 mmol, 96.27 %, colorless oil).
^{1}H NMR ($CDCl_3$): δ= 7.372-7.327 (3H, m), 7.190-6.863 (10H, m), 5.069 (2H, s), 4.947 (1H, brs), 4.031-3.995 (1H, m), 3.539-3.463 (3H, m), 3.317-3.260 (1H, m), 3.005 (2H, t, *J*= 7.6 Hz), 2.708 (2H, t, *J*= 7.6 Hz), 2.445 (1H, brs), 1.933-1.796 (2H, m), 1.724-1.640 (1H, m), 1.420-1.368 (1H, m).
^{13}C NMR ($CDCl_3$): δ= 173.76, 157.66, 156.92, 156.41, 139.29, 130.16, 129.97, 129.82, 128.83, 127.73, 123.49, 121.59, 120.85, 119.17, 117.95, 117.15, 111.63, 78.48, 69.34, 68.07, 67.03, 61.96, 34.18, 27.82, 26.29, 20.84.

HRMS (ESI-TOF $[M+Na]^+$) : Calcd. for $C_{28}H_{30}NaO_6^+$: 485.1935, Found 485.1923.
Anal. Calcd. for C, 69.17; H, 6.29; N, 0.00; (CH_2Cl_2 X 0.35). Found C, 69.07; H, 6.29; N, 0.00.

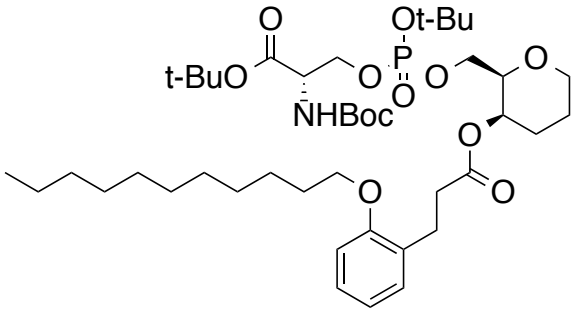
Compound **C-19**



Compound **A-3** (210.3 mg, 0.4656 mmol) and **C-16** (277.0 mg, 0.6984 mmol) were dissolved in CH_2Cl_2 (3 mL) and toluene (0.3 mL) and co-evaporated. The residue was dissolved in CH_2Cl_2 (3 ml) and *1H*-tetrazole (81.6 mg, 1.1642 mmol) in THF (3 mL) was added to the solution. The whole was stirred at room temperature under Ar atmosphere for 11 hrs. The reaction mixture was diluted with CH_2Cl_2 (10 mL) and quenched by saturated aqueous $NaHCO_3$ solution (10 mL). Water layer was extracted with CH_2Cl_2 (10 mL×2), washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography (hexane : ethyl acetate : NEt_3 = 35:4:1) to yield trivalent phosphodiester compound (230.6 mg, 0.3034 mmol, 65.17 %, colorless oil).
^{1}H NMR ($CDCl_3$): δ= 5.533-5.432 (1H, m), 5.390-5.292 (3H, m), 4.926 (1H, brs), 4.287-4.272 (1H, m), 4.128-3.975 (3H, m), 3.845-3.610 (3H, m), 3.534-3.3.474 (1H, m), 2.361-2.324 (2H, m), 2.054-1.837 (5H, m), 1.763-1.621 (4H, m), 1.535-1.192 (47H, m), 0.890-0.861 (3H, m).
^{31}P-NMR ($CDCl_3$): δ= 136.57. 135.44.
HRMS (ESI-TOF $[M+Na]^+$) : Calcd. for $C_{40}H_{74}NNaO_{10}P^+$: 782.4943, Found 782.4936.
Anal. Calcd. for C, 61.75; H, 9.85; N, 1.80; (H_2O×1). Found C, 62.05; H, 9.57; N, 0.00.

Trivalent phosphodiester compound obtained above was dissolved in CH_2Cl_2 (3 mL) and added *t*-butylhydroperoxide in decane (0.104 mL). The reaction mixture was stirred at room temperature under Ar atmosphere for 1 hr. After 1 hr, solvent was removed under vacuum and the residue was purified by column chromatography (hexane : acetone = 4:1) to yield **C-19** (190.8 mg, 0.2459 mmol, 94.29 %, colorless oil, (53 % two steps)).
^{1}H NMR ($CDCl_3$): δ= 5.516-5.464 (1H, m), 5.316-5.192 (3H, m), 4.866 (1H, brs), 4.298-4.262 (2H, m), 4.167-4.126 (1H, m), 3.996-3.840 (3H, m), 3.672-3.629 (1H, m), 3.474-3.414 (1H, m), 2.310-2.266 (2H, m), 1.992-1.767 (6H, m), 1.678-1.555 (3H, m), 1.463-1.165 (47H, m), 0.830-0.769 (3H, m).
^{13}C NMR ($CDCl_3$): δ= 173.02, 168.30, 155.26, 129.90, 129.65, 127.98, 127.84, 83.77, 83.69, 82.48, 82.43, 79.75, 76.62, 76.54, 76.44, 67.78, 67.72, 67.40, 66.65, 66.59, 66.53, 66.43, 66.38, 54.46, 54.38, 34.32. 34.31, 31.84, 31.82, 31.70, 29.73, 29.68, 29.65, 29.63, 29.56, 29.53, 29.44, 29.26, 29.23, 29,19, 29.10, 29.06, 28.27, 27.89, 27.88, 27.46, 27.39, 27.14, 17.12, 17.10, 24.97, 24.95, 22.59, 22.57, 20.53, 14.02.
^{31}P-NMR ($CDCl_3$): δ= -5.59, -5.89.
HRMS (ESI-TOF $[M+Na]^+$) : Calcd. for $C_{40}H_{74}NNaO_{11}P^+$: 798.4892, Found 798.4882.
Anal. Calcd. for C, 60.51; H, 9.65; N, 1.76; (H_2O×1). Found C, 60.90; H, 9.61; N, 1.76.

Compound **C-20**



Compound **A-3** (51.7 mg, 0.1146 mmol) and **C-17** (69.7 mg, 0.1604 mmol) was dissolved in CH_2Cl_2 (1 mL) and toluene (0.1 mL) and co-evaporated. The residue was dissolved in CH_2Cl_2 (1 mL) and *1H*-tetrazole (16.0 mg, 0.2291 mmol) in THF (1 mL) was added to the solution above. The whole was stirred at room temperature under Ar atmosphere for 15 hrs. After 15 hrs, the reaction mixture was diluted with CH_2Cl_2 (8 mL) and quenched by saturated aqueous $NaHCO_3$ solution (10 mL) and extracted with CH_2Cl_2 (8 mL×2). Combined organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography (hexane : ethyl acetate : NEt_3 = 35:4:1) to yield trivalent phosphodiester compound (58.1 mg, 0.0728 mmol, 45 %, colorless oil).

The trivalent phosphodiester compound (58.1 mg, 0.0728 mmol) was dissolved in CH_2Cl_2 (1 mL) tert-butylhydroperoxide in decane (0.0291 mL). The reaction mixture was stirred at room temperature under Ar atmosphere for 1 hr. After 1 hr, solvent was removed under vacuum and the residue was purified by column chromatography (hexane : ethyl acetate = 2:1) to yield **C-20** (44.6 mg, 0.0548 mmol, 75.27 %, colorless oil).

^{1}H NMR ($CDCl_3$): δ= 7.170-7.127 (2H, m), 6.851-6.796 (2H, m), 5.551-5.519 (1H, m), 4.905-4.898 (1H, m), 4.355-4.309 (2H, m), 4.225-4.171 (1H, m), 4.022-4.862 (5H, m), 3.709-3.667 (1H, m), 3.505-3.438 (1H, m), 2.977-2.885 (2H, m), 2.730-2.597 (2H, m), 1.986-1.951 (1H, m), 1.826-1.560 (27H, m), 1.363-1.231 (16H, m), 0.871 (3H, t, *J*= 7.0 Hz).

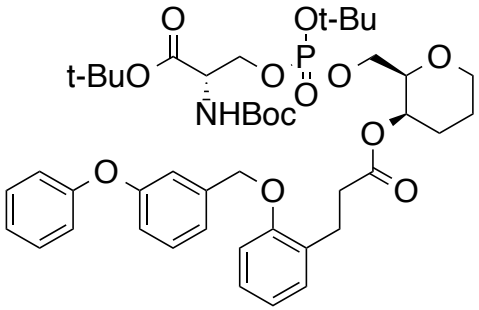
^{13}C NMR ($CDCl_3$): δ= 172.78, 168.39, 156.94, 155.33, 129.87, 128.68, 128.67, 127.53, 120.13, 110.98, 82.58, 82.55, 79.86, 76.58, 76.50, 67.82, 67.76, 67.51, 66.75, 66.63, 54.40, 34.14, 34.13, 31.90, 29.79, 29.75, 29.71, 29.64, 29.62, 29.60, 29.38, 29.34, 29.32, 28.33, 27.95, 27.50, 27.44, 26.18, 26.13, 22.67, 20.48, 14.11.

^{31}P-NMR ($CDCl_3$): δ= -5.50, -5.80.

HRMS (ESI-TOF $[M+Na]^+$) : Calcd. for $C_{42}H_{72}NNaO_{12}P^+$: 836.4684, Found 836.4712.

Anal. Calcd. for C, 61.97% ; H, 8.92%; N, 1.72%; [H_2O×1; C, 60.63% ; H, 8.96% ; N, 1.68%]; Found C, 60.61%; H, 8.56% ; N, 1.64%.

Compound **C-21**



Compound **A-3** (64.0 mg, 0.1417 mmol) and **C-18** (50.4 mg, 0.1090 mmol) was dissolved in CH_2Cl_2 (1 mL) and toluene (0.1 mL) and co-evaporated. The residue was dissolved

in CH_2Cl_2 (0.5 mL) and 1H-tetrazole(15.3 mg, 0.2179 mmol) in THF (0.5 mL) was added to the solution above. The whole was stirred at room temperature under Ar atmosphere for 20 hrs. After 20 hrs, the reaction mixture was quenched by saturated $NaHCO_3$ aqueous solution (10 mL) and extracted with CH_2Cl_2 (8 mL×2). Combined organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography (hexane : ethyl acetate : NEt_3 = 35:4:1) to yield trivalent phosphodiester compound (30.2 mg, 0.0366 mmol, 33.58 %).

The trivalent phosphodiester compound (30.2 mg, 0.0366 mmol) was dissolved in CH_2Cl_2 (1mL) tert-butylhydroperoxide in decane (0.0146 mL). The reaction mixture was stirred at room temperature under Ar atmosphere for 1 hr. After 1 hr, solvent was removed under vacuum and the residue waspurified by column chromatography (hexane : ethyl acetate = 2:1 to 1:1) to yield **C-21** (21.6 mg, 0.0257 mmol, 70.22 %, colorless oil).

^{1}H NMR ($CDCl_3$): δ= 7.361-7.311 (3H, m), 7.183-7.090 (4H, m), 7.053-6.998 (3H, m), 6.951-6.840 (3H, m), 5.553-5.524 (1H, m), 5.069 (2H, s), 4.899-4.892 (1H, m), 4.356-4.309 (2H, m), 4.226-4.171 (1H, m), 4.001-3.854 (3H, m), 3.707-3.662 (1H, m), 3.497-3.430 (1H, m), 3.039-2.927 (m, 2H), 2.738-2.603 (m, 2H), 1.968-1.933 (m, 1H), 1.799-1.592 (m, 3H), 1.460-1.312 (26H, m), 1.254-1.218 (1H, m).

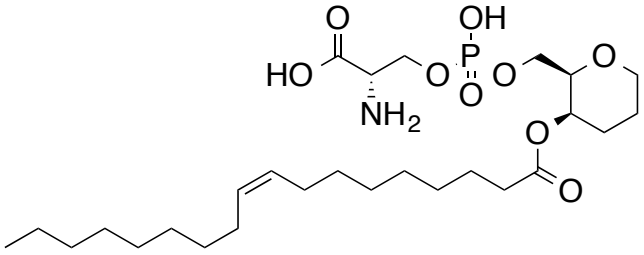
^{13}C NMR ($CDCl_3$): δ= 172.67, 168.39, 157.61, 156.92, 156.36, 139.33, 130.09, 129.96, 129.79, 128.93, 128.92, 127.60, 123.45, 121.53, 120.82, 119.14, 117.88, 117.08, 111.61, 82.59, 82.57, 79.88, 69.32, 67.82, 67.75, 66.82, 66.71, 54.50, 54.40, 34.10, 34.08, 29.79, 29.75, 29.71, 28.33, 27.95, 27.48, 27.43, 26.15.

^{31}P-NMR ($CDCl_3$): δ= -5.49, -5.79.

HRMS (ESI-TOF $[M+Na]^+$) : Calcd. for $C_{44}H_{60}NNaO_{13}P^+$: 864.3694, Found 864.3695.

Anal. Calcd. for C, 60.19; H, 7.35; N, 1.60; (H_2O×2). Found C, 60.17; H, 7.01; N, 1.54.

Compound **C-22**



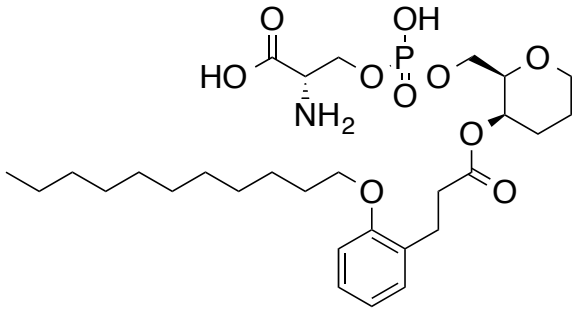
Compound **C-19** (101.8 mg, 0.1312 mmol) was dissolved in trifluoroacetic acid (2 mL) at 0 ℃ and stirred at room temperature for 3 hrs. After 3 hrs, solvent was removed under vacuum and the residue was purified by column chromatography ($CHCl_3$: MeOH: AcOH = 7:1:2 to 5:1:3) to yield **C-22** (22.7 mg, 0.0403 mmol, 30.7 %, white solid).

^{1}H NMR ($CDCl_3$): δ= 5.541-5.269 (1H, m), 5.191-5.065 (2H, m), 4.582-4.503 (3H, m), 4.206-4.184 (1H, m), 4.059-4.014 (2H, m), 3.893 (1H, brs), 3.691-3.632 (1H, m), 2.408-2.356 (2H, m), 2.060-1.966 (3H, m), 1.830-1.770 (1H, m), 1.652-1.607 (5H, m), 1.264 (21H, brs), 0.889-0.856 (3H, m).

^{31}P-NMR ($CDCl_3$): δ= -1.72.

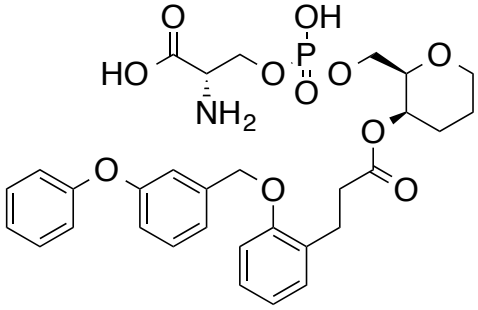
HRMS (ESI-TOF $[M-H]^-$) : Calcd. for $C_{27}H_{49}NO_{10}P^-$: 562.3150, Found 562.3192.

Compound **C-23**



Compound **C-20** (30.5 mg, 0.0375 mmol) was dissolved in trifluoroacetic acid (2 mL) at 0 ℃ and stirred at room temperature for 3.5 hrs. After 3.5 hrs, solvent was removed under vacuum and the residue was purified by column chromatography ($CHCl_3$: MeOH: AcOH = 6:1:2 to 5:1:4) to yield **C-23** (28.9 mg, 0.0480 mmol, 128.0 % (TFA salt), white solid).
^{1}H NMR ($CDCl_3$): δ= 7.215-7.176 (1H, m), 7.073-7.055 (1H, m), 6.900-6.856 (2H, m), 5.026 (1H, brs), 4.597-4.519 (3H, m), 4.173-4.147 (1H, m), 4.021-3.989 (4H, m), 3.870 (1H, brs), 3.655-3.600 (1H, m), 2.987-2.953 (2H, m), 2.882, 2.713 (2H, m), 1.893-1.689 (5H, m), 1.509-1.405 (3H, m), 1.363-1.270 (14H, m), 0.888-0.857 (3H, m).
^{31}P-NMR ($CDCl_3$): δ= -1.78.
HRMS (ESI-TOF: $[M-H]^-$): Calcd. for $C_{29}H_{47}NO_{10}P^-$: 600.2943, Found 600.2921.
Anal. Calcd. for C, 50.75; H, 7.01; N, 1.91; (H_2O×1, CF_3CO_2H×1). Found C, 50.53; H, 6.65; N, 1.90.

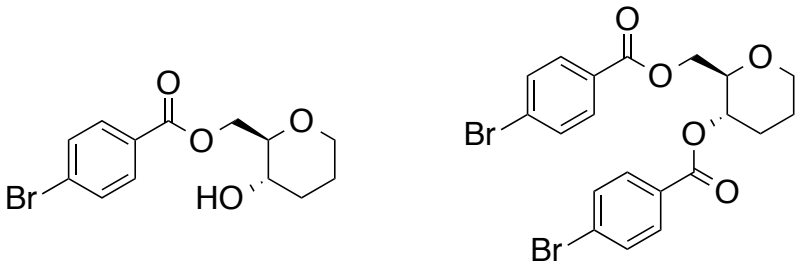
Compound **C-24**



Compound **C-21** (16.6 mg, 0.0197 mmol) was dissolved in trifluoroacetic acid (2 mL) at 0 ℃ and stirred at room temperature for 4.5 hrs. After 4,5 hrs, 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 **C-24** (8.3 mg, 0.0132 mmol, 66.92 % (TFA salt), white solid).
^{1}H NMR ($CDCl_3$): δ= 7.357-7.314 (3H, m), 7.206-7.065 (5H, m), 7.011-6.878 (5H, m), 5.802 (2H, s), 5.004 (1H, brs), 4.596 (2H, brs), 4.489 (1H, brs), 4.142-4.114 (1H, m), 3.989-3.975 (2H, m), 3.852-3.841 (1H, m), 3.629-3.577 (1H, m), 3.028-2.932 (2H, m), 2.810-2.711 (2H, m), 1.869-1.838 (1H, m), 1.778-1.640 (2H, m), 1.493-1.411 (1H, m).
^{31}P-NMR ($CDCl_3$): δ= -1.78.
HRMS (ESI-TOF: $[M-H]^-$): Calcd. for $C_{31}H_{35}NO_{11}P^-$: 628.1953, Found 628.1967.
Anal. Calcd. for C, 52.04; H, 5.16; N, 1.84; (H_2O×1, CF_3CO_2H×1). Found C, 52.12; H, 5.46; N, 1.81.

8. Examination of trans configuration

Compound **B-25 (left)**
Compound **B-26 (right)**



Compound **B-3** (60.8 mg, 0.4600 mmol) was dissolved in dry CH_2Cl_2 (3 mL) and cooled to 0 ℃. Triethylamine (232.8 mg, 2.300 mmol) was and *p*-bromobenzoylchloride (222.1 mg, 1.0121 mmol) was added to the solution above. The reaction mixture was stirred at room temperature under Ar atmosphere for 1.5 hrs. After 1.5 hrs, the reaction mixture was quenched by saturated NH_4Cl aqueous solution (2 mL) and the whole was washed with water (3 mL). Organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography (hexane : ethyl acetate = 2:1) to yield **B-26** (94.2 mg, 0.189 mmol, 41.1 %, white solid) and **B-25** (66.2 mg, 0.2101 mmol, 45.7 %, white solid).

B-25

^{1}H NMR ($CDCl_3$): δ= 7.872-7.838 (2H, m), 7.533-7.500 (2H, m), 4.449 (1H, dd, *J*= 11.6 Hz, 5.6 Hz), 4.361 (1H, dd, *J*= 11.6 Hz, 7.2 Hz), 4.016-3.975 (1H, m), 3.796 (1H, brs), 3.687-3.653 (1H, m), 3.499-3.434 (1H, m), 2.814 (1H, brs), 1.998-1.887 (2H, m), 1.719-1.629 (1H, m), 1.430-1.368 (1H, m).

^{13}C NMR ($CDCl_3$): δ= 166.90, 131.78, 131.75, 131.46, 131.42, 128.60, 128.48, 81.04, 68.05, 66.21, 65.08, 32.14, 25.38.

HRMS (ESI-TOF [M+Na]$^+$): Calcd. for $C_{13}H_{15}BrNaO_4^+$ 337.0046, Found 337.0047.

Anal. Calcd. for C, 49.54; H, 4.80; N, 0.00. Found C, 49.54; H, 4.61; N, 0.00.

B-26

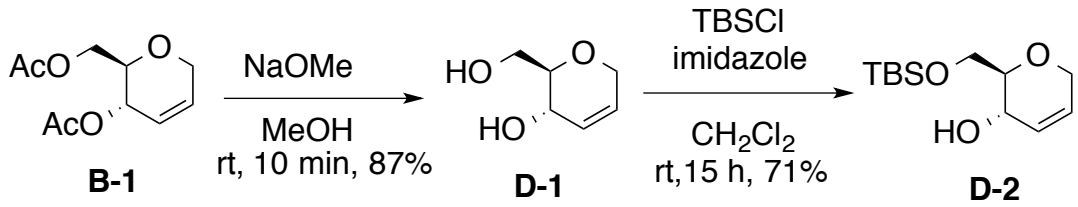
^{1}H NMR ($CDCl_3$): δ= 7.874-7.831 (4H, m), 7.558-7.508 (4H, m), 5.052-4.989 (1H, m), 4.569-4.532 (1H, dd, *J*= 12.0 Hz, 2.8 Hz), 4.380-4.336 (1H, dd, *J*= 12.0 Hz, 5.6 Hz), 4.044-4.004 (1H, m), 3.796-3.751 (1H, m), 3.507-3.442 (1H, dt, *J*= 11.6 Hz, 2.8 Hz), 2.392-2.352 (1H, m), 1.908-1.735 (2H, m), 1.686-1.584 (1H, m).

^{13}C NMR ($CDCl_3$): δ= 165.65, 164.77, 132.38, 131.92, 131.77, 131.65, 131.25, 131.14, 130.19, 128.80, 128.77, 128.39, 128.16, 127.54, 77.50, 69.49, 67.93, 64.55, 29.37, 24.94.

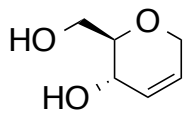
HRMS (ESI-TOF [M+Na]$^+$): Calcd. for $C_{20}H_{18}Br_2NaO_5^+$: 518.9413, Found 518.9417.

Anal. Calcd. for C, 48.22; H, 3.64; N, 0.00. Found C, 47.82; H, 3.59; N, 0.00.

9. Synthesis of modified unsaturated glycerol backbone

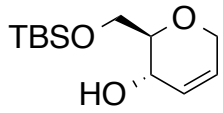


Compound **D-1**



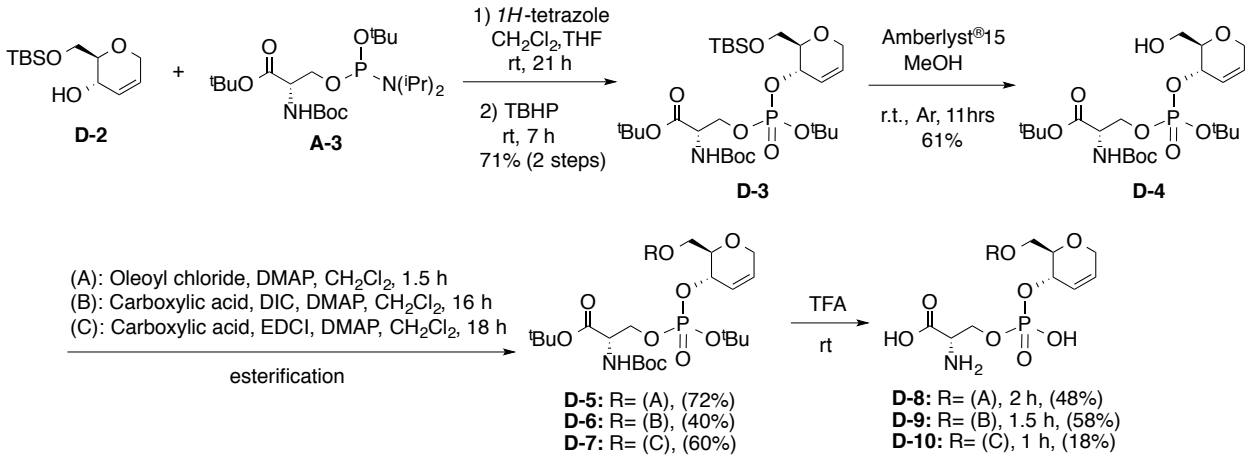
Compound **B-1** (784.5 mg, 3.6621 mmol) was dissolved in MeOH (10 mL) and sodium methoxide (98.9 mg, 1.8311 mmol) was added to the solution above. The reaction mixture was stirred at room temperature under Ar atmosphere for 10 min. After 10 min, solvent was evaporated and the residue was dissolved in chloroform. This solution was filtered on celite and the filtrate was evaporated to yield **D-1** (414.6 mg, 3.1858 mmol, 87.0 %, yellow oil).
^{1}H NMR ($CDCl_3$): δ= 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 ($CDCl_3$): δ= 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.

Compound **D-2**

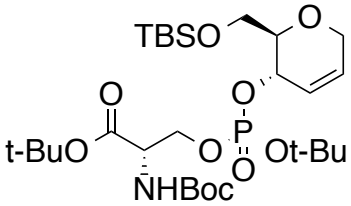


Compound **D-1** (445.6 mg, 3.4340 mmol) was dissolved in CH_2Cl_2 (10 mL) and imidiazole was added to the solution. This was cooled to 0℃ and *tert*-butyldimethylsilylchloride (567.7 mg, 3.7667 mmol) was added. This reaction mixture was stirred at 0 ℃ under Ar atmosphere for 10 min and stirred at room temperature for 15 hrs. After 15 hrs, water (20 mL) was added to the reaction mixture and the whole was extracted with CH_2Cl_2 (15 mL×3). Combined organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography to yield **D-2** (594.1 mg, 2.4309 mmol, 71.0 %, colorless oil).
^{1}H NMR ($CDCl_3$): δ= 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 ($CDCl_3$): δ= 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, 58.97; H, 9.90; N, 0.00. Found C, 58.89; H, 9.79; N, 0.00.

10. Synthesis of olefin-trans-1˚-acyl-2˚-phosphoserine



Compound **D-3**



A-3 (724.1 mg 1.6034 mmol) and compound **D-2** (587.8 mg, 2.4051 mmol) was dissolved in dry CH_2Cl_2 (10 mL) and dry toluene (2 mL) and co-evaporated 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 hrs. After 21 hrs, *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 hrs. After 7 hrs, water (20 mL) was added and the whole was extracted with CH_2Cl_2 (15 mL×2). Organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography (hexane : ethyl acetate = 4:1 to 2:1) to yield **D-3** (713.3 mg, 1.1435 mmol, colorless oil, 71.32 % 2 steps).

^{1}H NMR ($CDCl_3$): δ= 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).

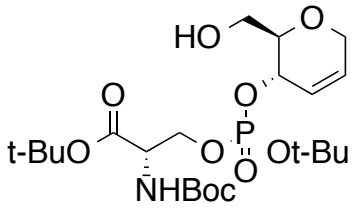
^{13}C NMR ($CDCl_3$): δ= 168.37, 168.32, 155.24, 129.86, 125.15, 125.01. 83.88, 83.81, 82.68, 79/92, 77.88, 77.78, 69.26, 69.20, 68.93, 68.87, 67.97, 67.66, 67.47, 64.95, 64.93, 63.08, 54.53, 54.45, 54.36, 30.91, 29.86, 29.84, 29.81, 29.80, 29.14, 28.33, 27.97, 25.98, 25.93, 23.91, 18.46, 18.45, 14.19.

^{31}P-NMR ($CDCl_3$): δ= -5.79, -5.86.

HRMS (ESI-TOF [M+Na]$^+$): Calcd. for $C_{28}H_{54}NNaO_{10}PSi^+$: 646.3147, Found 646.3151.

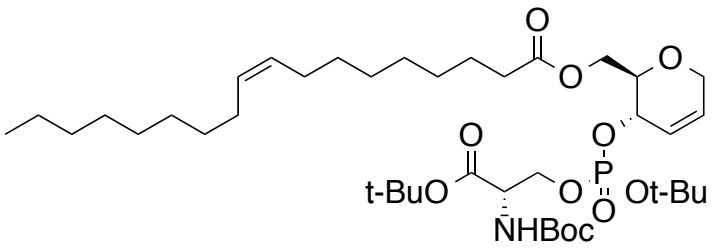
Anal. Calcd. for C, 53.38; H, 8.64; N, 2.22; (CH_2Cl_2X0.1). Found C, 53.11; H, 8.56; N, 2.24.

Compound **D-4**



Compound **D-3** (660.5 mg, 1.0588 mmol) was dissolved in MeOH (16 mL). Amberlyst®15 (3.3025 g) was added to the solution above and the reaction mixture was stirred at room temperature under Ar atmosphere for 11 hrs. After 11 hrs, the reaction mixture was filtered on celite and the filtrate was evaporated and the residue was purified by column chromatography (ethyl acetate) to yield **D-4** (322.2 mg, 0.6324 mmol, 60.67 %, white sticky solid).
^{1}H NMR ($CDCl_3$): δ= 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 ($CDCl_3$): δ= 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 ($CDCl_3$): δ= -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, 50.64; H, 7.73; N, 2.66; (CH_2Cl_2X0.2). Found C, 50.78; H, 7.74; N, 2.67.

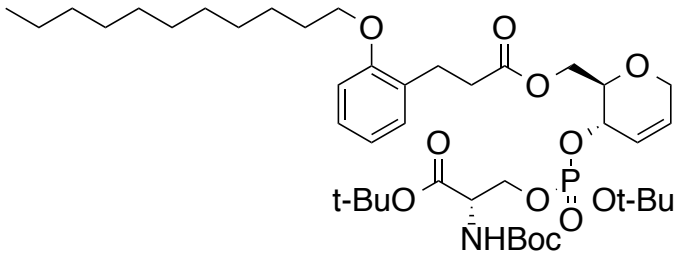
Compound **D-5**



Compound **D-4** (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 ℃ 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 hr. After 1.5 hrs, solvent was removed under vacuum and the residue was purified by column chromatography (hexane : ethyl acetate = 4:1 to 2:1) to yield **D-5** (101.1 mg, 0.1306 mmol, 71.65 %, colorless oil).
^{1}H NMR ($CDCl_3$): δ= 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 ($CDCl_3$): δ= 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,

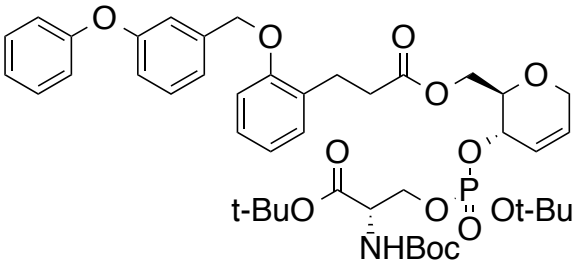
29.33, 29.31, 29.29, 29.28, 29.26, 29.17.29.16, 29.11, 29.10, 29.07, 28.95, 28.31, 27.95, 27.94, 27.19, 27.1625.60, 24.84, 22.64, 22.62, 22.54, 14.07, 14.04.
^{31}P-NMR ($CDCl_3$): δ= - 5.70, -5.78.
HRMS (ESI-TOF [M+Na]$^+$): Calcd. for $C_{40}H_{72}NNaO_{11}P^+$ 796.4735, Found 796.4730.
Anal. Calcd. for C, 61.04; H, 9.23; N, 1.77; (CH_2Cl_2X0.2). Found C, 61.02; H, 8.95; N, 1.82.

Compound **D-6**



Carboxylic acid derivatives (**B**) (39.1 mg, 0.1219 mmol), compound **D-4** (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 hrs. After 16 hrs, 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 (hexane : acetone = 3:1) to yield crude **D-6** (39.2 mg, 0.0483 mmol, 39.6 %, colorless oil).
^{1}H NMR ($CDCl_3$): δ= 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 ($CDCl_3$): δ= 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 ($CDCl_3$): δ= -5.48, -5.68.
HRMS (ESI-TOF [M+Na]$^+$) : Calcd. for $C_{42}H_{70}NNaO_{12}P^+$: 834.4533, Found 834.4522.

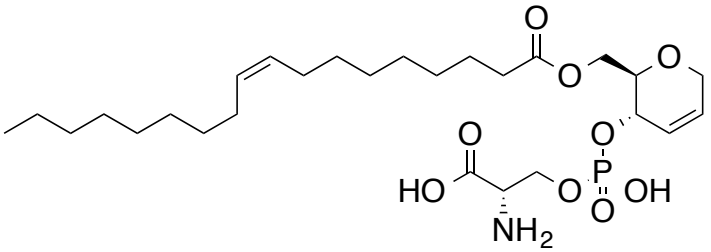
Compound **D-7**



Carboxylic acid derivative (**C**) (68.2 mg, 0.1958 mmol), compound **D-4** (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 hrs. After 18 hrs, the reaction mixture was quenched by water (8 mL) and extracted with CH_2Cl_2 (8 mL×2). Combined organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue

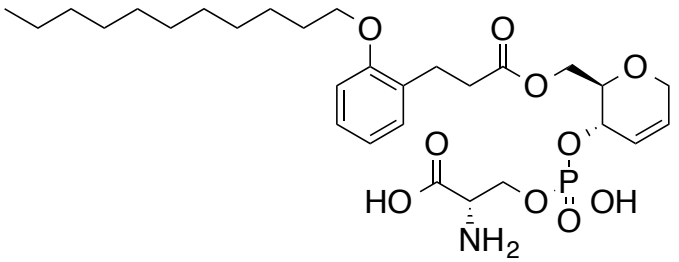
was purified by column chromatography (hexane : acetone = 3:1) to yield crude **D-7** (98.9 mg, 0.1178 mmol, 60.14 %, colorless oil).
^{1}H NMR ($CDCl_3$): δ=7.358-7.313 (3H, m), 7.191-7.084 (4H, m), 7.041-6.997 (3H, m), 6.951-6.926 (1H, m), 6.891-6.826 (2H, m), 5.920-5.910 (2H, m), 5.669-5.500 (1H, 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 ($CDCl_3$): δ= 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 ($CDCl_3$): δ=-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, 60.33; H, 7.13; N, 1.60. Found C, 60.12; H, 6.91; N, 1.51.

Compound **D-8**



Compoud **D-5** (98.8 mg, 0.1277 mmol) was dissolved in trifluoroacetic acid (2 mL) at 0 ℃, stirred at 0 ℃ for 10 min and stirred at room temperature for 2 hrs. After 2 hrs, 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 **D-8** (34.6 mg, 0.0616 mmol, 48.2 %, white solid).
^{1}H NMR ($CDCl_3$): δ= 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 ($CDCl_3$): δ= -2.72.
HRMS (ESI-TOF: $[M-H]^-$): Calcd. for $C_{27}H_{47}NO_9P^-$: 560.2994, Found 560.3027.

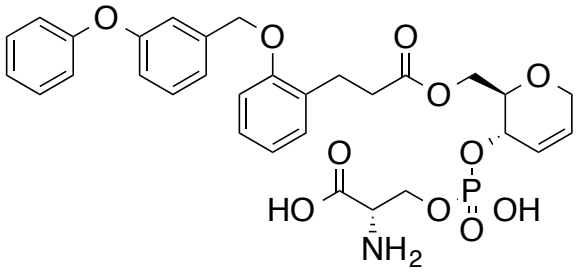
Compound **D-9**



Compound **D-6** (35.5 mg, 0.0437 mmol) was dissolved in trifluoroacetic acid (2 mL) at 0 ℃ and stirred at room temperature for 1.5 hrs. After 1.5 hrs, 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 **D-9** (15.3 mg, 0.0255mmol, 58.39 %, white solid, (20.9 %, 2 steps)).

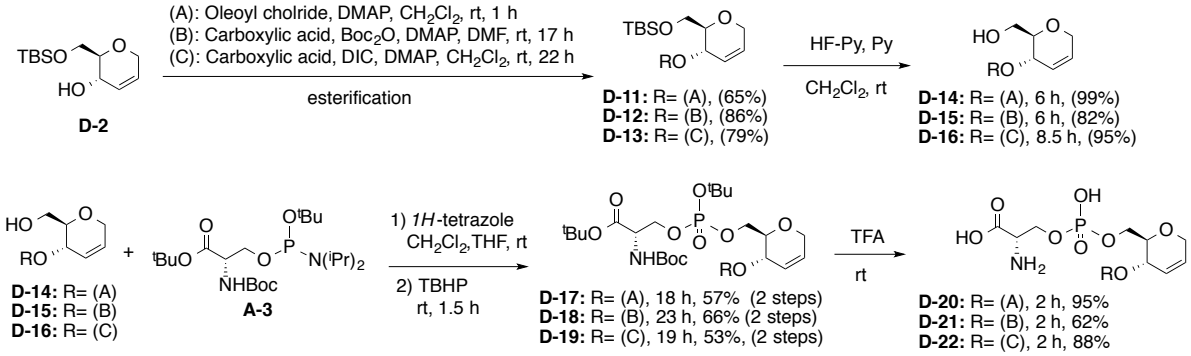
^{1}H NMR ($CDCl_3$): δ= 7.214-7.176 (1H, m), 7.076-7.059 (1H, m), 6.895-6.829 (2H, m), 6.027-6.003 (1H, m), 5.876-5.809 (1H, m), 4.738-4.424 (5H, m), 4.310-4.254 (3H, m), 4.011-3.977 (2H, m), 3.864-3.861 (1H, m), 2.951-2.866 (2H, m), 2.773-2.756 (2H, m), 1.816-1.746 (2H, m), 1.427-1.267 (16H, m), 0.890-0.857 (3H, m).
^{31}P-NMR ($CDCl_3$): δ= -1.85.
HRMS (ESI-TOF [M-H]$^-$) : Calcd. for $C_{29}H_{49}NO_{10}P^-$: 598.2787, Found 598.2779.
Anal. Calcd. for C, 49.87; H, 6.17; N, 1.82; ($CF_3CO_2HX1.5$). Found C, 49.72; H, 6.10; N, 1.68.

Compound **D-10**

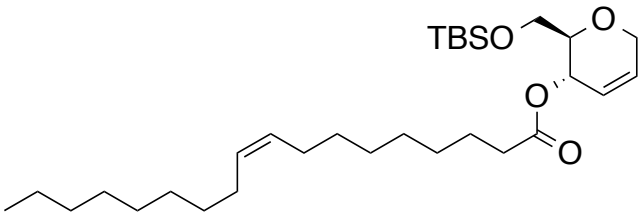


Compound **D-7** (98.0 mg, 0.1167 mmol) was dissolved in trifluoroacetic acid (2 mL) at 0 ℃ and stirred at room temperature for 1 hrs. After 1 hrs, solvent was removed under vacuum and the residue was purified by column chromatography ($CHCl_3$: MeOH : AcOH = 6:1:2 to 4:1:3) to yield **D-10** (12.9 mg, 0.0206 mmol, 17.7 %, white solid (10.5 %, 2 steps)).
^{1}H NMR ($CDCl_3$): δ= 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 ($CDCl_3$): δ= -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, 48.12; H, 4.38; N, 1.60; (CF_3CO_2HX2/H_2OX1). Found C, 48.15; H, 4.26; N, 1.78.

11. Synthesis of olefin-trans-2˚-acyl-1˚-phosphoserine

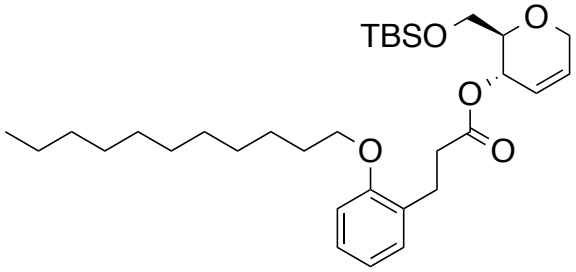


Compound **D-11**



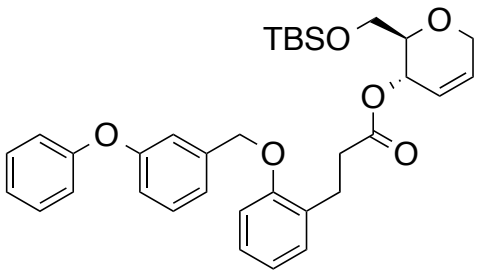
Compound **D-2** (92.9 mg, 0.3801 mmol) was dissolved in CH_2Cl_2 (1.5 mL) and *N,N*-dimethylaminopyridine (139.3 mg,1.1403 mmol) was added to the solution. Oleoyl chloride (137.3 mg, 0.4561 mmol) was added under Ar. The reaction mixture was stirred at room temperature under Ar for 1 hr. After 1 hrs, solvent was removed under vacuum and the residue was purified by column chromatography (hexane : ethyl acetate = 20:1) to yield **D-11** (125.0 mg, 0.2457 mmol, 64.6 %, colorless oil).
^{1}H NMR ($CDCl_3$): δ= 5.938-5.896 (1H, m), 5.767-5.724 (1H, m), 5.382-5.294 (2H, m), 5.263-5.220 (1H, m), 4.202-4.183 (2H, m), 3.778-3.686 (2H, m), 3.581-3.540 (1H, m), 2.317-2.280 (2H, m), 2.052-1.980 (4H, m), 1.634-1.580 (2H, m), 1.298-1.262 (20H, m), 0.899-0.858 (12H, m), 0.061-0.606 (6H, m).
^{13}C NMR ($CDCl_3$): δ= 173.17, 130.01, 129.73, 129.66, 124.55, 76.88, 65.34, 64.97, 63.26, 34.45, 31.91, 29.77, 29.70, 29.53, 29.33, 29.17, 29.11, 29.09, 27.22, 27.17, 25.96, 24.96, 22.69, 18.46, 14.12, -5.27, -5.33.
HRMS (ESI-TOF $[M+Na]^+$) : Calcd. for $C_{30}H_{56}NaO_4Si^+$: 531.3846, Found 531.3852.
Anal. Calcd. for C, 69.95; H, 10.88; N, 0.00; (CH_2Cl_2×0.1). Found C, 70.20; H, 10.78; N, 0.00.

Compound **D-12**



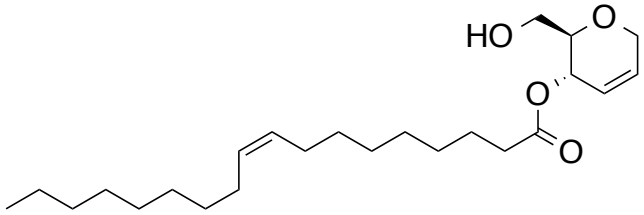
Carboxylic acid derivative (**B**) (130.7 mg, 0.407 mmol) and compound **D-2** (99.7 mg, 0.4079 mmol) was dissolved in CH_2Cl_2 (2 mL) and diisopropylcarbodiimide (66.9 mg, 0.5303 mmol) and *N,N*-dimethylaminopyridine (10 mg, 0.0816 mmol) were added to the solution above. The whole was stirred at room temperature under Ar atmosphere for 17 hrs. After 17 hrs, the reaction mixture was diluted with CH_2Cl_2 (8 mL) and quenched by water (8 mL). Water layer was extracted with CH_2Cl_2 (8 mL×2), combined organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography (hexane : ethyl acetate = 20:1) to yield **D-12** (192.0 mg, 0.3511 mmol, 86.1 %, colorless oil).
^{1}H NMR ($CDCl_3$): δ= 7.198-7.138 (2H, m), 6.879-6.817 (2H, m), 5.927-5.884 (1H, m), 5.748-5.705 (1H, m), 5.287-5.245 (1H, m), 4.199-4.189 (2H, m), 3.982-3.949 (2H, m), 3.759-3.668 (2H, m), 3.757-3.533 (1H, m), 2.976-2.937 (2H, m), 2.662-2.624 (2H, m), 1.843-1.773 (2H, m), 1.502-1.446 (2H, m), 1.384-1.287 (14H, m), 0.910-0.881 (12H, m), 0.070-0.069 (6H, m).
^{13}C NMR ($CDCl_3$): δ= 172.72, 156.97, 129.96, 129.50, 128.69, 127.60, 124.71, 120.19, 111.00, 77.40, 67.72, 65.41, 65.03, 63.24, 34.30, 31.95, 29.67, 29.65, 29.42, 29.38, 29.36, 26.26, 26.19, 25.98, 22.71, 18.47, 14.15.
HRMS (ESI-TOF $[M+Na]^+$) : Calcd. for $C_{32}H_{54}NaO_5Si^+$: 569.3633, Found 569.3647.
Anal. Calcd. for C, 70.28; H, 9.95; N, 0.00. Found C, 70.07; H, 9.91; N, 0.00.

Compound **D-13**



Carboxylic acid derivative (**C**) (74.5 mg, 0.2138 mmol) and compound **D-2** (52.3 mg, 0.2138 mmol) was dissolved in CH_2Cl_2 (1 mL) and diisopropylcarbodiimide (35.1 mg, 0.2780 mmol) and *N,N*-dimethylaminopyridine (5.2 mg, 0.0428 mmol) were added to the solution above. The whole was stirred at room temperature under Ar atmosphere for 22 hrs. After 22 hrs, the reaction mixture was diluted with CH_2Cl_2 (7 mL) and quenched by water (7 mL). Water layer was 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 (hexane : ethyl acetate = 8:1 to 4:1) to yield **D-13** (97.2 mg, 0.1691mmol, 79.1 %, colorless oil).
^{1}H NMR ($CDCl_3$): δ= 7.388-7.342 (3H, m), 7.208-7.168 (3H, m), 7.158-7.116 (1H, m), 7.094-7.090 (1H, m), 7.061-7.031(2H, m), 6.984-6.960 (1H, m), 6.936-6.870 (2H, m), 5.922-5.880 (1H, m), 5.737-5.695 (1H, m), 5.290-5.248 (1H, m), 5.081 (2H, s), 4.240-4.116 (2H, m), 3.753-3.663 (2H, m), 3.583-3.542 (1H, m), 3.005 (2H, t, *J*= 7.6 Hz), 2.672-2.633 (2H, m), 0.912 (9H, s), 0.068 (6H, s).
^{13}C NMR ($CDCl_3$): δ= 172.63, 157.71, 156.91, 156.40, 139.32, 130.21, 129.96, 129.84, 129.60, 128.96, 127.69, 124.62, 123.53, 121.54, 120.88, 119.22, 117.92, 117.11, 111.61, 76.83, 69.32, 65.45, 64.99, 63.22, 34.28, 26.20, 26.00, 18.49, -5.21, -5.29.
HRMS (ESI-TOF $[M+Na]^+$) : Calcd. for $C_{34}H_{42}NaO_6Si^+$: 597.2643, Found 597.2670.
Anal. Calcd. for C, 71.05; H, 7.37; N, 0.00. Found C, 70.09; H, 7.30; N, 0.00.

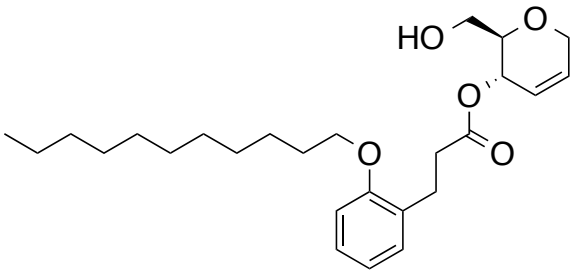
Compound **D-14**



Compound **D-11** (119.7 mg, 0.2352 mmol) was dissolved in THF (1.5 mL) and hydrogen fluoride-pyridine complex (123.1 μL) in pyridine (303.1 μL) was added to the solution above. This reaction mixture was stirred at room temperature under Ar atmosphere for 6 hrs. After 6 hrs, the reaction mixture was diluted with CH_2Cl_2 (7 mL) and water (7 mL) was added to the solution. Water layer was extracted with CH_2Cl_2 (7 mL×2), combined organic layer was washed with brine, dried over $MgSO_4$ and solvent was evaporated. The residue was purified by column chromatography (hexane : ethyl acetate = 2:1) to yield **D-14** (91.6 mg, 0.2321 mmol, 98.7 %, colorless oil).
$^{1-}$H NMR ($CDCl_3$): δ= 6.104-6.066 (1H, m), 5.960-5.910 (1H, m), 5.356-5.274 (2H, m), 5.135-5.122 (1H, m), 4.344-4.288 (1H, m), 4.213-4.155 (1H, m), 3.719-3.696 (2H, m), 3.590-3.551 (1H, m), 2.474 (1H, brs), 2.340-2.302 (2H, m), 2.005-1,957 (4H, m), 1.618-1.583 (2H, m), 1.273-1.240 (21H, m), 0.869-0.835 (3H, m).
^{13}C NMR ($CDCl_3$): δ= 174.03, 132.53, 129.98, 129.69, 122.29, 76.82, 65.84, 64.31, 61.82, 34.25, 31.89, 29.75, 29.66, 29.50, 29.30, 29.12, 29.07, 27.20, 27.13, 24.96, 22.66, 14.10.
HRMS (ESI-TOF $[M+Na]^+$) : Calcd. for $C_{24}H_{42}NaO_4^+$: 417.2981, Found 417.2962.

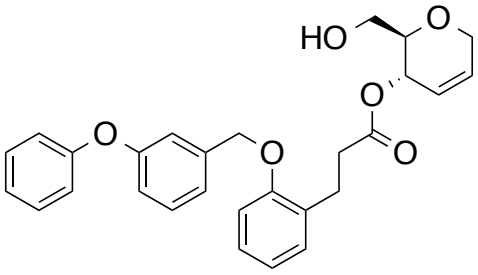
Anal. Calcd. for C, 72.11; H, 10.67; N, 0.00; (H_2O×0.3). Found C, 72.34; H, 10.49; N, 0.00.

Compound **D-15**



Compound **D-12** (177.3 mg, 0.3242 mmol) was dissolved in THF (2 mL) and hydrogen fluoride-pyridine complex (182.3 μL) in pyridine (449.0 μL) was added to the solution above. This reaction mixture was stirred at room temperature under Ar atmosphere for 6 hrs. After 6 hrs, the reaction mixture was diluted with CH_2Cl_2 (15 mL) and water (15 mL) was added to the solution. Water layer was extracted with CH_2Cl_2 (10 mL×2), combined organic layer was washed with brine, dried over $MgSO_4$ and solvent was evaporated. The residue was purified by column chromatography (hexane : ethyl acetate = 2:1) to yield **D-15** (115.5 mg, 0.2670 mmol, 82.4 %, colorless oil).
^{1}H NMR ($CDCl_3$): δ= 7.196-7.121 (2H, m), 6.872-6.811(2H, m), 5.917-5.873 (1H, m), 5.721-5.679 (1H, m), 5.309-5.278 (1H, m), 4.218-4.199 (2H, m), 3.956 (2H, t, *J*= 6.5 Hz), 3.653-3.614 (1H, m), 3.511-3.440 (2H, m), 2.981-2.927 (2H, m), 2.676-2.638 (2H, m), 1.830-1.761 (2H, m), 1.504-1.432 (2H, m), 1.349-1.273 (14H, m), 0.902-0.867 (3H, m).
^{13}C NMR ($CDCl_3$): δ= 173.22, 156.98, 129.99, 129.11, 128.47, 127.69, 120.17, 111.03, 76.23, 67.74, 65.32, 65.16, 62.18, 34.22, 31.94, 29.66, 29.40, 29.37, 29.33, 26.32, 26.19, 22.71, 14.15.
HRMS (ESI-TOF [M+Na]$^+$) : Calcd. for $C_{26}H_{40}NaO_5^+$: 455.2768, Found 455.2766.
Anal. Calcd. for C, 72.19; H, 9.32; N, 0.00. Found C, 71.90; H, 9.21; N, 0.00.

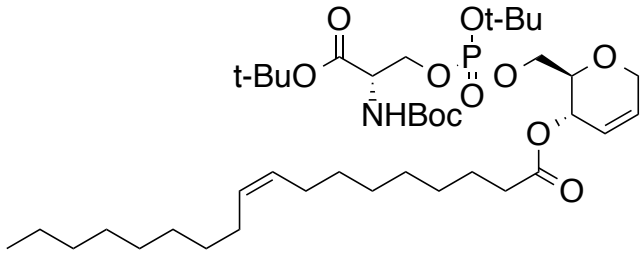
Compound **D-16**



Compound **D-13** (93.7 mg, 0.1630 mmol) was dissolved in THF (1.2 mL) and hydrogen fluoride-pyridine complex (85.3 μL) in pyridine (210 μL) was added to the solution above. This reaction mixture was stirred at room temperature under Ar atmosphere for 8.5 hrs. After 8.5 hrs, the reaction mixture was diluted with CH_2Cl_2 (10 mL) and water (10 mL) was added to the solution. Water layer was extracted with CH_2Cl_2 (10 mL×2), combined organic layer was washed with brine, dried over $MgSO_4$ and solvent was evaporated. The residue was purified by column chromatography (hexane : ethyl acetate = 2:1) to yield **D-16** (71.1 mg, 0.1544 mmol, 94.7 %, colorless oil).
^{1}H NMR ($CDCl_3$): δ= 7.378-7.332 (3H, m), 7.213-7.107 (4H, m), 7.086 (1H, m), 7.052-7.021 (2H, m), 6.975-6.950 (1H, m), 6.930-6.870 (2H, m), 5.9-6-5.863 (1H, m), 5.708-5.666 (1H, m), 5.307-277 (1H, m), 5.073 (2H, s), 4.207-4.188 (2H, m), 3.631-3.592 (1H, m), 3.809-3.434 (2H, m), 3.011-2.974 (2H, m), 2.682-2.644 (2H, m), 2.278 (1H, brs).

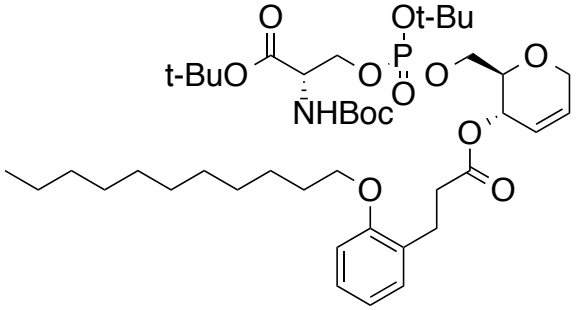
^{13}C NMR ($CDCl_3$): δ= 173.09, 157.71, 156.90, 156.41, 139.29, 130.23, 129.96, 129.84, 129.18, 128.75, 127.78, 124.84, 123.54, 121.56, 120.88, 119.21, 117.93, 117.13, 111.65, 76.17, 69.33, 65.31, 65.22, 62.18, 53.48, 34.21, 26.29.
HRMS (ESI-TOF [M+Na]$^+$) : Calcd. for $C_{28}H_{28}NaO_6^+$: 483.1778, Found 483.1783.
Anal. Calcd. for C, 73.03; H, 6.13; N, 0.00. Found C, 72.80; H, 6.24; N, 0.00.

Compound **D-17**



Compound **A-3** (68.2 mg, 0.1510 mmol) and **D-14** (89.4 mg, 0.2266 mmol) was dissolved in CH_2Cl_2 (1 mL) and toluene (0.2 mL) and co-evaporated. The residue was dissolved in CH_2Cl_2 (1 mL) and *1H*-tetrazole (31.7 mg, 0.4531 mmol) in THF (1 mL) was added to the solution above. The whole was stirred at room temperature under Ar atmosphere for 18 hrs. After 18 hrs, the reaction mixture was diluted with CH_2Cl_2 (8 mL) and quenched by saturated $NaHCO_3$ aqueous solution (8 mL) and extracted with CH_2Cl_2 (8 mL×2). 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 to 2:1) to yield crude trivalent phosphodiester compound (83.2 mg, 0.1098 mmol, 72.7 %, colorless oil).
The trivalent phosphodiester compound (83.1 mg, 0.1096 mmol) was dissolved in CH_2Cl_2 (1 mL) tert-butylhydroperoxide in decane (0.0439 mL). The reaction mixture was stirred at room temperature under Ar atmosphere for 1.5 hr. After 1.5 hr, solvent was removed under vacuum and the residue was purified by column chromatography (hexane : ethyl acetate = 4:1 to 2:1) to yield **D-17** (66.5 mg, 0.0859 mmol, 78.4 %, colorless oil, 56.9 %(2steps)).
^{1}H NMR ($CDCl_3$): δ= 5.923-5.897 (1H, m), 5.735-5.707 (1H, m), 5.623-5.563 (1H, m), 5.356-5.276 (2H, m), 5.209-5.152 (1H, m), 4.359-4.333 (2H, m), 4.212-4.194 (3H, m), 4.098-4.977 (2H, m), 3.l732-3.687 (1H, m), 2.314-2.276 (2H, m), 1.988-1.956 (4H, m), 1.840-1.807 (1H, m), 1.609-1.576 (2H, m), 1.471-1.419 (26H, m), 1.274-1.238 (20H, m), 0.867-0.833 (3H, m).
^{13}C NMR ($CDCl_3$): δ= 173.08, 168.41, 168.37, 155.33, 129.97, 129.70, 129.60, 129.51, 124.23, 124.06, 83.91, 82.53, 82.48, 79.78, 74.59, 74.52, 67.94, 67.57, 67.52, 66.32, 66.24, 66.18, 64.91, 64.83, 64.64, 54.49, 54.40, 34.28, 31.88, 29.78, 29.74, 29.67, 29.50, 29.29, 29.13, 29.07, 28.33, 27.95, 27.93, 27.19, 27.14, 25.59, 24.86, 24.84, 22.66, 14.09.
^{31}P NMR ($CDCl_3$): δ= -5.64, -5.88.
HRMS (ESI-TOF [M+Na]$^+$) : Calcd. for $C_{40}H_{72}NaO_{11}P^+$: 796.4741, Found 796.4713.
Anal. Calcd. for C, 62.07; H, 9.38; N, 1.81. Found C, 61.87; H, 9.27; N, 1.86.

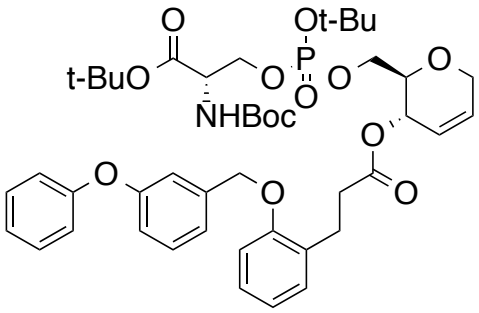
Compound **D-18**



Compound **A-3** (79.5 mg, 0.1769 mmol) and **D-15** (114.2 mg, 0.2634 mmol) was dissolved in CH_2Cl_2 (1.2 mL) and toluene (0.1 mL) and co-evaporated. The residue was dissolved in CH_2Cl_2 (1.2 mL) and *1H*-tetrazole (24.7 mg, 0.3520 mmol) in THF (1.2 mL) was added to the solution above. The whole was stirred at room temperature under Ar atmosphere for 23 hrs. After 23 hrs, the reaction mixture was diluted with CH_2Cl_2 (10 mL) and quenched by saturated $NaHCO_3$ aqueous solution (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 (hexane : ethyl acetate = 4:1 to 2:1) to yield crude trivalent phosphodiester compound (96.4 mg, 0.1211 mmol, 68.8 %, colorless oil).

The trivalent phosphodiester compound (96.3 mg, 0.1210 mmol) was dissolved in CH_2Cl_2 (1 mL) tert-butylhydroperoxide in decane (0.0484 mL). The reaction mixture was stirred at room temperature under Ar atmosphere for 1.5 hr. After 1.5 hr, solvent was removed under vacuum and the residue was purified by column chromatography (hexane : ethyl acetate = 2:1 to 1:1) to yield **D-18** (94.6 mg, 0.1165 mmol, 96.3 %, colorless oil, 65.9 %(2 steps)).
^{1}H NMR ($CDCl_3$): δ= 7.170-7.104 (2H, m), 6.849-6.791 (2H, m), 5.911-5.882 (1H, m), 5.691-5.588 (2H, m), 5.215-5.156 (1H, m), 4.366-4.340 (2H, m), 4.249-4.128 (3H, m), 4.069-3.874 (m, 4H), 3.759-3.658 (m, 1H), 2.984-2.865 (m, 2H), 2.675-2.595 (m, 2H), 1.813-1.743 (2H, m), 1.480-1.426 (28H, m), 1.351-1.221 (15H, m), 0.881-0.847 (3H, m).
^{13}C NMR ($CDCl_3$): δ= 172.67, 168.44, 168.40, 156.93, 155.35, 129.97, 129.94, 129.49, 129.39, 128.51, 127.63, 127.60, 124.29, 124.11, 120.16, 111.01, 83.87, 83.80, 82.52, 82.46, 79.77, 74.58, 74.51, 67.73, 67.56, 67.50, 66.30, 66.23, 66.17, 64.87, 64.59, 54.51, 54.43, 34.12, 31.90, 29.79, 29.75, 29.73, 29.62, 29.37, 29.33, 29.30, 28.33, 27.96, 27.94, 26.15, 26.13, 22.67, 14.11.
^{31}P NMR ($CDCl_3$): δ= -5.64, -5.92.
HRMS (ESI-TOF $[M+Na]^+$) : Calcd. for $C_{42}H_{70}NNaO_{12}P^+$: 834.4528, Found 834.4503.
Anal. Calcd. for C, 62.13; H, 8.69; N, 1.75. Found C, 61.99; H, 8.39; N, 1.76.

Compound **D-19**

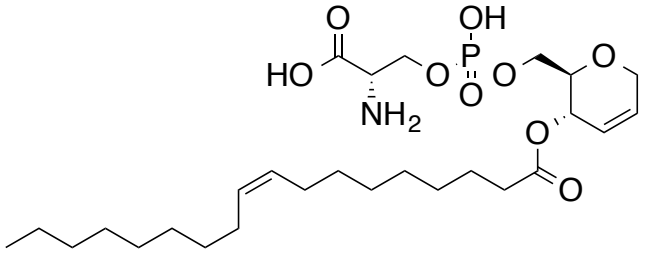


Compound **A-3** (46.4 mg, 0.1028 mmol) and **D-16** (71.0 mg, 0.1563 mmol) was dissolved in CH_2Cl_2 (1 mL) and toluene (0.2 mL) and co-evaporated. The residue was dissolved in CH_2Cl_2 (1 mL) and *1H*-tetrazole (14.4 mg, 0.2056 mmol) in THF (1 mL) was added to the solution above. The whole was stirred at room temperature under Ar atmosphere for 19 hrs. After 19 hrs, the reaction mixture was diluted with CH_2Cl_2 (8mL) and quenched by saturated $NaHCO_3$ aqueous solution (8 mL) and extracted with CH_2Cl_2 (8 mL×2). 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 to 2:1) to yield crude trivalent phosphodiester compound (57.1 mg, 0.0693 mmol, 67.42 %, colorless oil, 154 sm recovery : 35.4 mg).

The trivalent phosphodiester compound (57.1 mg, 0.0693 mmol) was dissolved in CH_2Cl_2 (1 mL) *tert*-butylhydroperoxide in decane (0.0277 mL). The reaction mixture was stirred at room temperature under Ar atmosphere for 1 hr. After 1 hr, solvent was removed

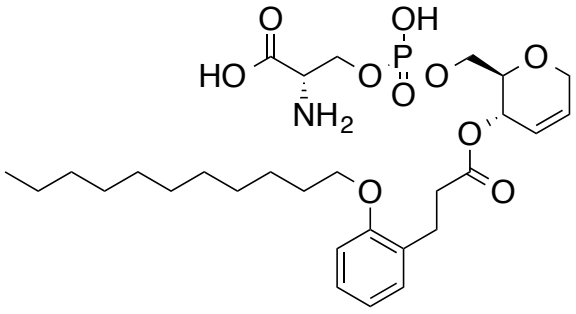
under vacuum and the residue was purified by column chromatography (hexane : ethyl acetate = 2:1 to 1:1) to yield **D-19** (46.1 mg, 0.0549 mmol, 79.25 %, colorless oil, 53.4 %(2 steps)).
^{1}H NMR ($CDCl_3$): δ= 7.358-7.313 (3H, m), 7.175-7.086 (4H, m), 7.054-6.994 (3H, m), 6.948-6.840 (3H, m), 5.899-5.869 (1H, m), 5.668-5.602 (2H, m), 5.210-5.152 (1H, m), 5.061 (2H, s), 4.365-4.336 (2H, m), 4.251-4.180 (3H, m), 4.061-3.933 (2H, m), 3.717-3.652 (1H, m), 3.028-2.909 (2H, m), 2.678-2.598 (2H, m), 1.479-1.429 (27H, m).
^{13}C NMR ($CDCl_3$): δ= 172.56, 168.46, 168.42, 157.66, 156.87, 156.35, 155.37, 139.31, 139.29, 130.21, 130.17, 129.93, 129.81, 129.54, 129.44, 128.82, 128.76, 127.71, 127.68, 124.23, 124.05, 123.49, 121.50, 120.85, 119.17, 117.87, 117.07, 111.61, 83.91, 82.54, 82.48, 79,80, 74.53, 74.46, 69.30, 67.52, 66.27, 66.21, 66.14, 64.89, 64.57, 54.50, 54.42, 53.44, 34.10, 29.80, 29.76, 28.34, 27.97, 27.95, 26.13, 26.11, 14.20.
^{31}P NMR ($CDCl_3$): δ= -5.62, -5.90.
HRMS (ESI-TOF $[M+Na]^+$) : Calcd. for $C_{44}H_{58}NNaO_{13}P^+$: 862.3538, Found 862.3526.
Anal. Calcd. for C, 58.44; H, 6.54; N, 1.51; (CH_2Cl_2×1). Found C, 58.54; H, 6.49; N, 1.59.

Compound **D-20**



Compound **D-17** (74.1 mg, 0.095 7mmol) was dissolved in TFA (1.5 mL) at 0 °C and stirrted at 0 °C for 15 min and at room temperature for 2 hrs. The reaction mixture was evaporated and the residue was purified by column chromatography ($CHCl_3$: MeOH : H_2O = 65:25:4) to yield **D-20** (51.1 mg, 0.0910 mmol, 95.1 %, white solid).
^{1}H NMR ($CDCl_3$): δ (ppm): δ = 6.022-5.996 (1H, m), 5.725-5.699 (1H, m), 5.481-5.422 (1H, m), 5.397-5.303 (1H, m), 5.104-5.043 (1H, m), 4.584 (2H, m), 4.477 (1H, m), 4.38904.244 (2H, m), 4.144 (1H, m), 4.054 (1H, m), 3.781 (1H, m), 2.375-2.361 (2H, m), 2.018-1.965 (2H, m), 1.645-1.585 (4H, m), 1.264 (20H, m), 0.887-0.853 (3H, m).
^{31}P-NMR ($CDCl_3$): δ (ppm): δ = -2.57.
HRMS (ESI-TOF $[M+Na]^+$) : Calcd. for $C_{27}H_{47}NO_9P^-$: 560.2994, Found 560.3006.
Anal. Calcd. for C, 46.42; H, 6.18; N, 1.72; (CF3CO2H×2.2). Found C, 46.20; H, 6.16; N, 1.81.

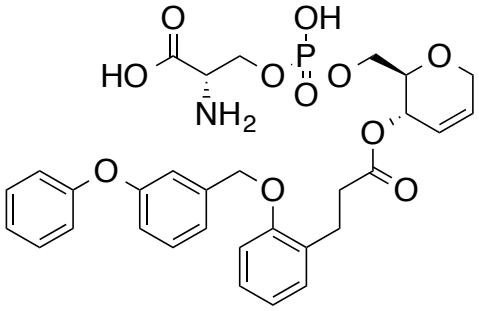
Compound **D-21**



Compound **D-18** (94.1 mg, 0.1159 mmol) was dissolved in TFA (1.5 mL) at 0°C and stirrted at 0 °C for 10 min and at room temperature for 2 hrs. The reaction mixture was evaporated and the residue was purified by column chromatography ($CHCl_3$: MeOH: H_2O = 65:25:4) to yield **D-21**

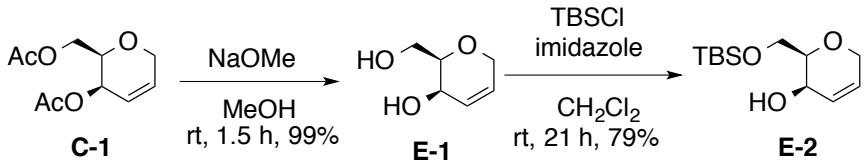
(42.9 mg, 0.0715 mmol, 61.7 %, white solid, TFA salt: 45.1 mg, 64.9 % /crude 34.2 mg, 49.2 %).
^{1}H NMR ($CDCl_3$): δ= 7.210-7.146 (1H, m), 7.098-6.996 (1H, m), 6.914-6.803 (2H, m), 5.963-5.938 (1H, m), 5.568-5.542 (1H, m), 5.399 (1H, m), 4.572 (2H, m), 4.453 (1H, m), 4.254 (2H, m), 4.075 (1H, m), 4.016-3.962 (3H, m), 3.689 (1H, m), 2.981-2.914 (2H, m), 2.786-2.735 (2H, m), 1.815-2.745 (2H, m), 1.490-1.416 (2H, m), 1.299-1.267 (14H, m), 0.893-0.859 (3H, m).
^{31}P NMR ($CDCl_3$): δ= -2.36.
HRMS (ESI-TOF [M-H]$^-$) : Calcd. for $C_{29}H_{45}NO_{10}P^-$: 598.2787, Found 598.2763.
Anal. Calcd. for C, 43.23; H, 3.53; N, 1.29: (CF_3CO_2H×4). Found C, 43.06; H, 3.86; N, 1.58.

Compound **D-22**

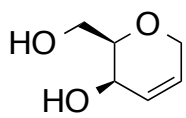


Compound **D-19** (46.0 mg, 0.0548 mmol) was dissolved in TFA (1 mL) at 0 °C and stirrted at 0 °C for 10 min and at room temperature for 2 hrs. The reaction mixture was evaporated and the residue was purified by column chromatography ($CHCl_3$: MeOH : H_2O = 65:25:4) to yield **D-22** (30.1 mg, 0.0480 mmol, 87.5 %, white solid).
^{1}H NMR ($CDCl_3$): δ= 7.361-7.304 (3H, m), 7.210-7.167 (1H, m), 7.144-7.105 (2H, m), 7.074-7.060 (2H, m), 7.014-6.984 (2H, m), 6.956-6.931 (2H, m), 6.904-6.869 (2H, m), 5.917-5.891 (1H, m), 5.513-5.484 (1H, m), 5.408-5.392 (1H, m), 5.072 (2H, s), 4.568 (2H, s), 4.442 (1H, m), 4.278-4.214 (2H, m), 4.077-4.053 (1H, m), 3.956-3.943 (1H, m), 3.664-3.648 (1H, m), 3.115-2.902 (2H, m), 2.796-2.612 (2H, m).
^{31}P NMR ($CDCl_3$): δ= -2.47.
HRMS (ESI-TOF [M-H]$^-$): Calcd. for $C_{31}H_{33}NO_{11}P^-$: 626.1797, Found 626.1825.
Anal. Calcd. for C, 43.23; H, 3.53; N, 1.29: (CF_3CO_2H×4). Found C, 43.06; H, 3.86; N, 1.58.

12. Synthesis of modified unsaturated glycerol backbone (cis)

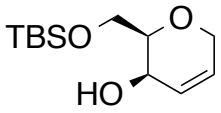


Compound **E-1**



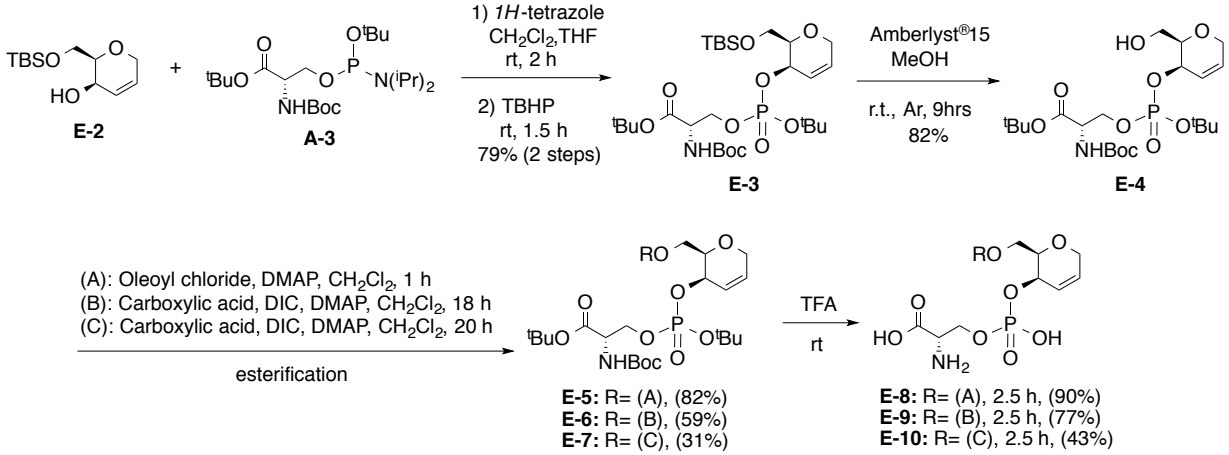
Compound **C-1** (543.0 mg, 2.5348 mmol) was dissolved in methanol (5 mL), sodium methoxide (68.5 mg, 1.2674 mmol) was added to the solution above. The reaction mixture was stirred at room temperature under Ar for 1.5 h. After 1.5 h, the reaction mixture was evaporated to remove solvent and the residue was dissolved in chloroform. The solution was filtered on celite and filtrate was evaporated to yield crude **E-1** (329.3 mg, 2.5301 mmol, 99.8 %, colorless sticky oil).
^{1}H NMR ($CDCl_3$): δ= 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 ($CDCl_3$): δ= 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.

Compound **E-2**

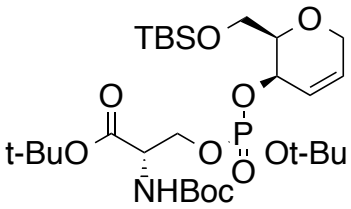


Compound **E-1** (460.6 mg, 3.5347 mmol) was dissolved in CH_2Cl_2 (10 mL) and imidazole (529.4 mg, 7.7762 mmol) was added to the solution above. This solution was cooled to 0 ℃ and *tert*-butyldimethylsilylchloride (639.3 mg, 4.2416 mmol) was added. The reaction mixture was stirred at 0 ℃ under Ar for 10 min and stirred at room temperature under Ar for 21 hrs. The reaction mixture was quenched by water (20 mL) and the whole was extracted with CH_2Cl_2 (15 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 = 1:1 to ethyl acetate) to yield **E-2** (275.2 mg, 1.1260 mmol, 79 %, colorless oil).
^{1}H NMR ($CDCl_3$): δ= 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 ($CDCl_3$): δ= 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 C, 58.97; H, 9.90; N, 0.00. Found C, 58.88; H, 9.60; N, 0.00.

13. Synthesis of olefin-cis-1˚-acyl-2˚-phosphoserine

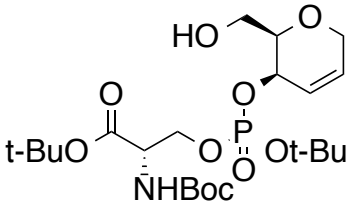


Compound **E-3**



A-3 (319.8 mg, 0.7081 mmol) and compound **E-2** (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 hrs. After 2 hrs, *tert*-butylhydroperoxide (0.2832 mg, 1.4162 mmol) was added to the reaction mixture and stirred at room temperature under Ar for 1.5 hrs. After 1.5 hrs, 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 (hexane : ethyl acetate = 2:1) to yield **E-3** (349.6 mg, 0.5604 mmol, 79.14 % (2 steps), colorless oil).
^{1}H NMR ($CDCl_3$): δ= 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 ($CDCl_3$): δ= 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 ($CDCl_3$): δ= -5.56, -5.82.
HRMS (ESI-TOF [M+Na]$^+$): Calcd. for $C_{28}H_{54}NNaO_{10}P^+$: 646.3147, Found 646.3160.
Anal. Calcd. for C, 53.91; H, 8.73; N, 2.25. Found C, 53.64; H, 8.44; N, 2.35.

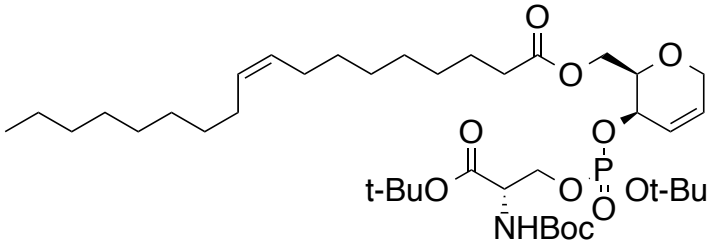
Compound **E-4**



Compound **E-3** (285.4 mg, 0.4575 mmol) was dissolved in methanol (7 mL) and this solution was cooled to 0 ℃. Amberlyst®15 (1.4110 g) was added to the solution above, stirred at 0 ℃ under Ar for 5 min and stirred at room temperature under Ar for 9 hrs. After 9 hrs, reaction mixture was filtered on celite and the filtrate was evaporated. The residue was purified by column chromatography (ethyl acetate) to yield **E-4** (191.6 mg, 0.3769 mmol, 82.2 %, colorless oil).
^{1}H NMR ($CDCl_3$): δ= 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 ($CDCl_3$): δ= 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.

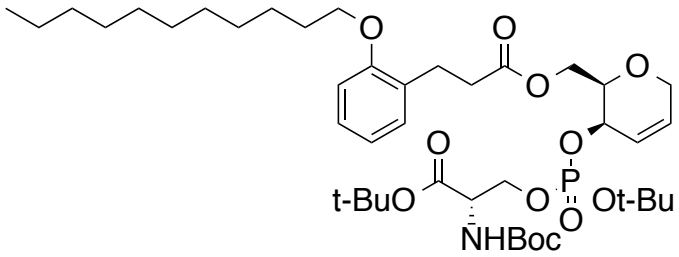
^{31}P-NMR ($CDCl_3$): δ= - 3.39, -3.69.
HRMS (ESI-TOF [M+Na]$^+$): Calcd. for $C_{22}H_{40}NNaO_{10}P^+$: 532.2282, Found 532.2298.

Compound **E-5**



Compound **E-4** (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 ℃ and oleoyl chloride (89.1 mg, 0.2962 mmol) was added under Ar. The reaction mixture was stirred at 0 ℃ under Ar for 10 min and stirred at room temperature under Ar for 1 hr. After 1 hrs, solvent was removed under vacuum and the residue was purified by column chromatography (hexane : ethyl acetate = 2:1) to yield **E-5** (125.4 mg, 0.1620 mmol, 82.1 %, colorless oil).
^{1}-H NMR ($CDCl_3$): δ= 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 ($CDCl_3$): δ= 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 ($CDCl_3$): 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, 62.07; H, 9.38; N, 1.8. Found C, 61.88; H, 9.35; N, 1.79.

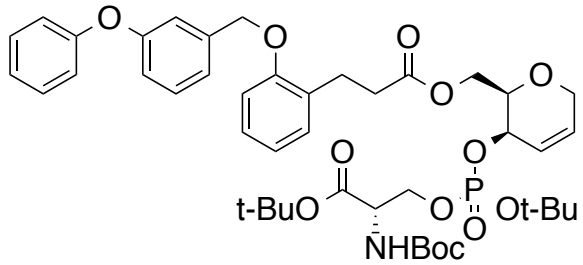
Compound **E-6**



Carboxylic acid derivatives (**B**) (67.7 mg, 0.2111 mmol), compound **E-4** (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 hrs. After 18 hrs, 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 : CH2Cl2 : MeOH = 12:1, 2nd : hexane : acetone =

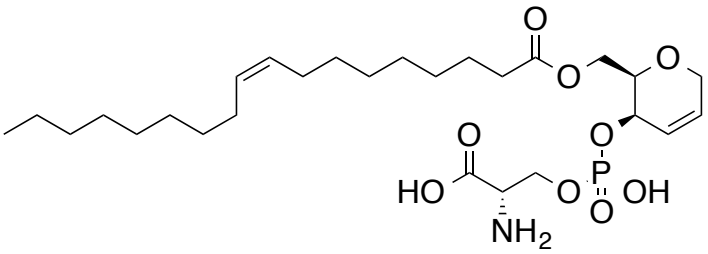
5:1, 3rd : hexane : ethyl acetate = 2:1) to yield **E-6** (101.0 mg, 0.1244 mmol, 58.92 %, colorless oil).
^{1}H NMR ($CDCl_3$): δ= 7.174-7.127 (2H, m), 6.854-6.800 (2H, m), 6.104-6.039 (2H, m), 5.630-5.475 (1H, m), 4.590-4.579 (1H, m), 4.381-4.106 (7H, m), 3.947 (2H, t, J = 6.4 Hz), 3.765-3.732 (1H, 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 ($CDCl_3$): δ=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 ($CDCl_3$): δ= -5.54, -5.72.
HRMS (ESI-TOF $[M+Na]^+$) : Calcd. for $C_{42}H_{70}NNaO_{12}P^+$: 834.4533, Found 834.4530.

Compound **E-7**



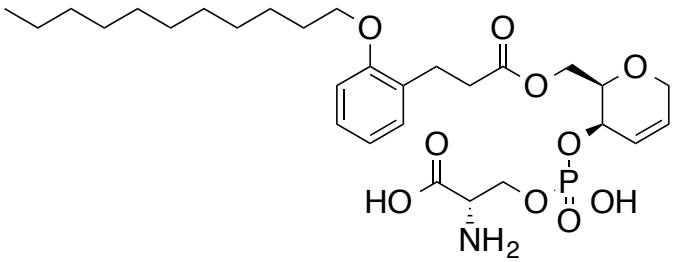
Carboxylic acid derivative (**C**) (59.0 mg, 0.1693 mmol), compound **E-4** (86.2 mg, 0.1693 mmol) were dissolved in CH_2Cl_2 (2 mL) and diisopropylcarbodiimide (47.0 mg, 0.3724 mmol) and *N,N*-dimethylaminopyridine (2.1 mg, 0.0169 mmol) were added to the solution above. The mixture was stirred at room temperature under Ar atmosphere for 20 hrs. After 20 hrs, 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 (hexane : acetone = 3:1) to yield crude **E-7** (43.4 mg, 0.0517 mmol, 30.5 %, colorless oil).
^{1}H NMR ($CDCl_3$): δ= 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 ($CDCl_3$): δ= 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 ($CDCl_3$): δ= -5.52, -5.72.
HRMS (ESI-TOF $[M+Na]^+$) : Calcd. for $C_{44}H_{58}NNaO_{13}P^+$: 862.3538, Found 862.3520.

Compound **E-8**

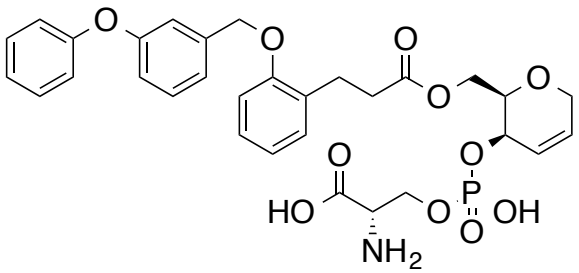


Compoud **E-5** (114.8 mg, 0.1483 mmol) was dissolved in trifluoroacetic acid (2 mL) at 0 ℃, stirred at 0 ℃ for 10 min and stirred at room temperature for 2.5 hrs. After 2.5 hrs, the reaction mixture was evaporated to remove solvent and the residue was purified by column chromatography ($CHCl_3$:MeOH: AcOH = 7:1:1 to 6:1:4) to yield **E-8** (74.8 mg, 0.1332 mmol, 89.8 %, white solid).
^{1}H NMR ($CDCl_3$): δ= 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 ($CDCl_3$): δ= -2.66.
HRMS (ESI-TOF: $[M-H]^-$): Calcd. for $C_{27}H_{47}NO_9P^-$: 560.2994, Found 560.2991.
Anal. Calcd. for C, 50.21; H, 7.41; N, 2.02; (CF_3CO_2HX1/H_2OX1). Found C, 50.95; H, 7.33; N, 2.01

Compound **E-9**

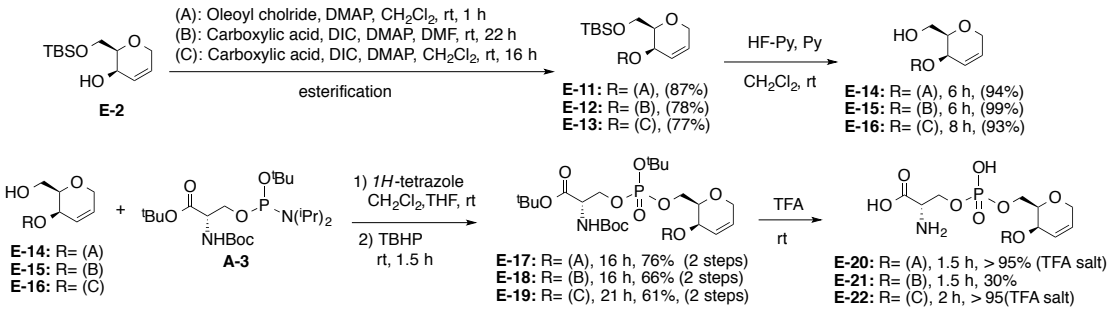


Compound **E-6** (91.1 mg, 0.1122 mmol) was dissolved in trifluoroacetic acid (2 mL) at 0 ℃ and stirred at room temperature for 2.5 hrs. After 2.5 hrs, solvent was removed under vacuum and the residue was purified by column chromatography ($CHCl_3$: MeOH : AcOH = 6:1:2 to 5:1:3) to yield **E-9** (52.1 mg, 0.0869 mmol, 77.4 %, white solid).
^{1}H-NMR ($CDCl_3$): δ= 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 ($CDCl_3$): δ= -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, 53.20; H, 6.78; N, 2.03; ($CF_3CO_2HX0.8$). Found C, 53.47; H, 6.84; N, 2.12.
Compound **E-10**

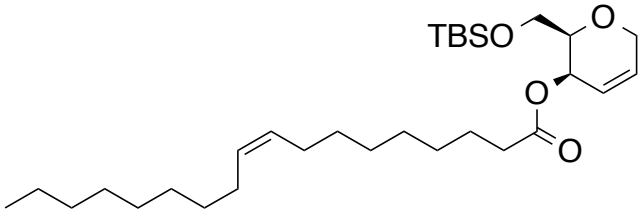


Compound **E-7** (43.1 mg, 0.0513 mmol) was dissolved in trifluoroacetic acid (1 mL) at 0 ℃ and stirred at room temperature for 2.5 hrs. After 2.5 hrs, solvent was removed under vacuum and the residue was purified by column chromatography ($CHCl_3$: MeOH : AcOH = 6:1:2 to 5:1:3) to yield **E-10** (13.7 mg, 0.0128 mmol, 42.6 %, white solid (8 %, 2 steps)).
^{1}H NMR ($CDCl_3$): δ= 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 ($CDCl_3$): δ= -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, 49.13; H, 4.24; N, 1.64. Found C, 48.78; H, 4.24; N, 1.61.

14. Synthesis of olefin-cis-2˚-aycl-1˚-phosphoserine

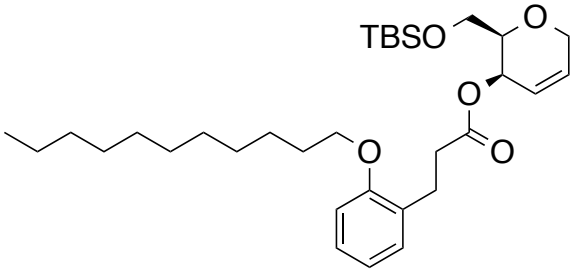


Compound **E-11**



Compound **E-2** (92.9 mg, 0.3801 mmol) was dissolved in CH_2Cl_2 (1 mL) and *N*,*N*-dimethylaminopyridine (103.0 mg, 0.8433 mmol) was added to the solution. Oleoyl chloride (101.5 mg, 0.3373 mmol) was added under Ar. The reaction mixture was stirred at room temperature under Ar for 1 hr. After 1 hrs, solvent was removed under vacuum and the residue was purified by column chromatography (hexane : ethyl acetate = 20:1) to yield **E-11** (123.8 mg, 0.2433 mmol, 86.6 %, colorless oil).
^{1}H NMR ($CDCl_3$): δ= 6.059-5.997 (2H, m), 5.364-5.278 (2H, m), 5.115-5.094 (1H, m), 4.300-4.255 (1H, m), 4.178-4.135 (1H, m), 3.754-3.667 (3H, m), 2.302 (2H, t, *J*= 7.6 Hz), 2.013-1.965 (2H, m), 1.621-1.567 (2H, m), 1.280-1.250 (20H, m), 0.860 (12H, m), 0.039 (3H, s), 0.030 (3H, s).
^{13}C NMR ($CDCl_3$): δ=173.19, 132.15, 129.95, 129.72, 122.77, 76.65, 65.85, 63.95, 61.9234.29, 31.90, 29.76, 29.70, 29.52, 29.31, 29.18, 29.12, 29.09, 27.20, 27,17, 25.81, 24.99, 22.67, 18.19, 14.10.
HRMS (ESI-TOF [M+Na]$^+$) : Calcd. for $C_{30}H_{56}NaO_4Si^+$: 531.3846, Found 531.3860.
Anal. Calcd. for C, 70.81; H, 11.09; N, 0.00. Found C, 70.60; H, 10.79; N, 0.00.

Compound **E-12**



Carboxylic acid derivative (**B**) (120.4 mg, 0.3756 mmol) and compound **E-2** (91.8 mg, 0.3756 mmol) was dissolved in CH_2Cl_2 (2 mL) and diisopropylcarbodiimide (61.6 mg, 0.4883 mmol) and *N,N*-dimethylaminopyridine (9.2 mg, 0.0751 mmol) were added to the solution above. The whole was stirred at room temperature under Ar atmosphere for 22 hrs. After 22 hrs, the reaction mixture was diluted with CH_2Cl_2 (8 mL) and quenched by water (8 mL). Water layer was extracted with CH_2Cl_2 (8 mL×2), combined organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography (hexane : ethyl acetate = 10:1 to 5:1) to yield **E-12** (160.2 mg, 0.2930 mmol, 78.0 %, colorless oil).

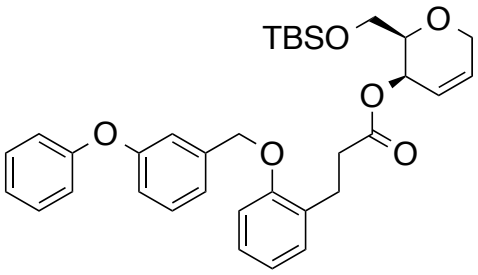
^{1}H NMR ($CDCl_3$): δ= 7.185-7.143 (2H, m), 6.868-6.806 (2H, m), 6.078-6.021 (2H, m), 5.136 (1H, m), 4.316-4.276 (1H, m), 4.190-4.119 (1H, m), 3.970-3.937 (2H, m), 3.756-3.690 (3H, m), 2,990-2.935 (2H, m), 2.678-2.640 (2H, m), 1.837-1.744 (2H, m), 1.490-1.435 (2H, m), 1.355-1.248 (14H, m), 0.914-0.880 (12H, m), 0.060 (2H, s), 0.044 (3H, s).

^{13}C NMR ($CDCl_3$): δ= 172.83, 156.98, 132.13, 129.97, 128.87, 127.49, 122.82, 120.15, 110.93, 76.71, 67.73, 65.84, 64.13, 61.96, 34.10, 31.95, 29.68, 29.66, 29.62, 29.43, 19.38, 29.36, 26.16, 25.99, 25.84, 22.72, 22.54, 18.54, 18.21, 17.88, 14.16, -5.18, -5.34.

HRMS (ESI-TOF $[M+Na]^+$) : Calcd. for $C_{32}H_{54}NaO_5Si^+$: 569.3633, Found 569.3605.

Anal. Calcd. for C, 70.28; H,9.95; N, 0.00. Found C, 70.04; H, 9.77; N, 0.00.

Compound **E-13**

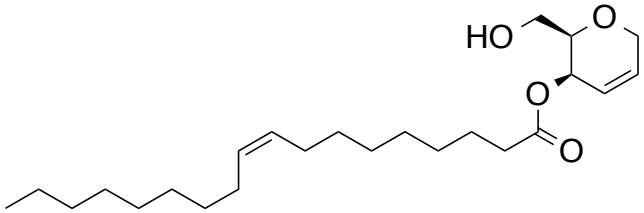


Carboxylic acid derivative (**C**) (79.4 mg, 0.2279 mmol) and compound **E-2** (55.7 mg, 0.2279 mmol) was dissolved in CH_2Cl_2 (1.7 mL) and diisopropylcarbodiimide (37.4 mg, 0.2963 mmol) and *N,N*-dimethylaminopyridine (5.6 mg, 0.0456 mmol) were added to the solution above. The whole was stirred at room temperature under Ar atmosphere for 16 hrs. After 16 hrs, the reaction mixture was diluted with CH_2Cl_2 (10 mL) and quenched by water (10 mL). Water layer 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 (hexane : ethyl acetate = 8:1 to 4:1 to 2:1) to yield **E-13** (101.0 mg, 0.1757 mmol, 77.1 %, colorless oil, sm 40 recovery :11.5 mg).

^{1}H NMR ($CDCl_3$): δ= 7.386-7.340 (3H, m), 7.209-7.111 (4H, m), 7.069-7.030 (3H, m), 6.982-6.957 (1H, m), 6.992-6.850 (2H, m), 6.071-6.013 (2H, m), 5.143 (1H, m), 5.072 (2H, s), 4.323-

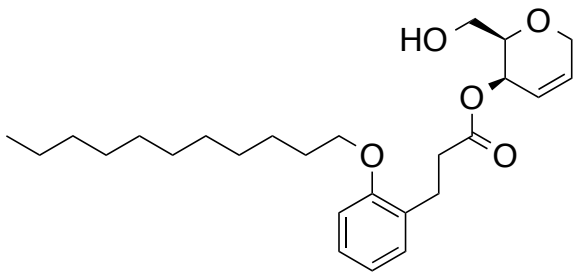
4.280 (1H, m), 4.200-4.159 (1H, m), 3.737-3.683 (3H, m), 3.063-2.949 (2H, m), 2.734-2.612 (2H, m), 0.889 (9H, s), 0.058 (3H, s), 0.035 (3H, s).
^{13}C NMR ($CDCl_3$): δ= 172,74, 157.67, 156.94, 156.41, 139.35, 132.19, 130.22, 129.99, 129.83, 129.54, 129.12, 127.58, 123.50, 122.78, 121.56, 120.84, 119.22, 117.92, 117.08, 111.57, 76.71, 69.31, 65.86, 64.21, 61.99, 60.41, 34.06, 26.10, 25.86, 18.23, 17.91, -5.17, -5.31, -5.45.
HRMS (ESI-TOF [M+Na]$^+$) : Calcd. for $C_{34}H_{42}NaO_6Si^+$: 597.2643, Found 597.2658.
Anal. Calcd. for C, 71.05; H,7.37; N, 0.00. Found C, 70.86; H, 7.29; N, 0.00.

Compound **E-14**



Compound **E-11** (122.3 mg, 0.2403 mmol) was dissolved in THF (1.7 mL) and hydrogen fluoride-pyridine complex (125.8 μL) in pyridine (309.7 μL) was added to the solution above. This reaction mixture was stirred at room temperature under Ar atmosphere for 6 hrs. After 6 hrs, the reaction mixture was diluted with CH_2Cl_2 (7 mL) and water (7 mL) was added to the solution. Water layer was extracted with CH_2Cl_2 (7 mL×2), combined organic layer was washed with brine, dried over $MgSO_4$ and solvent was evaporated. The residue was purified by column chromatography (hexane : ethyl acetate = 2:1) to yield **E-14** (89.2 mg, 0.2261 mmol, 94.1 %, colorless oil).
^{1}H NMR ($CDCl_3$): δ= 6.104-6.066 (1H, m), 5.960-5.910 (1H, m), 5.356-5.274 (2H, m), 5.135-5.122 (1H, m), 4.344-4.288 (1H, m), 4,213-4.155 (1H, m), 3,719 (2H, m), 3.590-3.551 (1H, m), 2.474 (1H, brs), 2.340-2.302 (2H, m), 2.005-1.957 (4H, m), 1.618-1.583 (2H, m), 1.273-1.240 (20H, m), 0.869-0.835 (3H, m).
^{13}C NMR ($CDCl_3$): δ=174.03, 132.53, 129.98, 129.69, 122.29, 76.82, 65.84, 64.31, 61.82, 34.25, 31.89, 29.75, 29.66, 29.50, 29.30, 29.12, 29.07, 27.20, 37.14, 24.96, 22.66, 14.10.
HRMS (ESI-TOF [M+Na]$^+$) : Calcd. for $C_{24}H_{42}NaO_4^+$: 417.2981, Found 417.2974.
Anal. Calcd. for C, 72.43; H, 10.66; N, 0.00; (H_2O×0.2). Found C, 72.29; H, 10.55; N, 0.00.

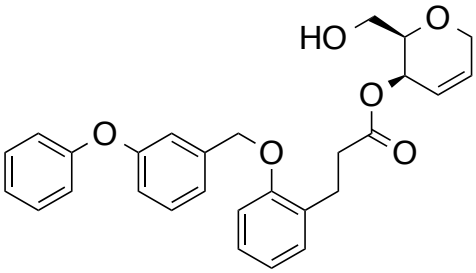
Compound **E-15**



Compound **E-12** (157.8 mg, 0.2886 mmol) was dissolved in THF (2 mL) and hydrogen fluoride-pyridine complex (151.0 μL) in pyridine (371.8 μL) was added to the solution above. This reaction mixture was stirred at room temperature under Ar atmosphere for 6 hrs. After 6 hrs, the reaction mixture was diluted with CH_2Cl_2 (10 mL) and water (10 mL) was added to the solution. Water layer was extracted with CH_2Cl_2 (10 mL×2), combined organic layer was washed with brine, dried over $MgSO_4$ and solvent was evaporated. The residue was purified by

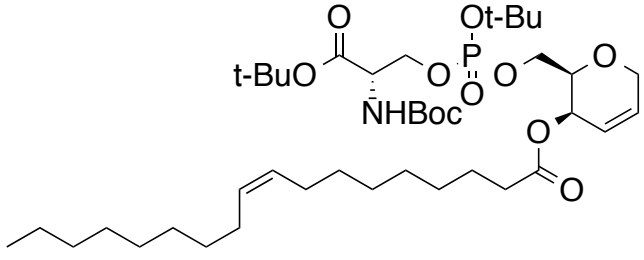
column chromatography (hexane : ethyl acetate = 2:1 to 1:1) to yield **E-15** (123.1 mg, 0.2846 mmol, 98.6 %, colorless oil).
^{1}H NMR ($CDCl_3$): δ= 7.188-7.120 (2H, m), 6.862-6.805 (2H, m), 6.097-6.059 (1H, m), 5.956-5.906 (1H, m), 5.134-5.110 (1H, m), 4.311 (dddd, 1H, *J*= 11.6 Hz, 3.6 Hz, 2.0 Hz, 1.6 Hz), 4.107-1.149 (1H, m), 3.963-3.830 (2H, m), 3.719-3.683 (1H, m), 3.659-3.612 (1H, m), 3.465-3.424 (1H, m), 3.009-2.890 (2H, m), 2.740-2.618 (2H, m), 2.385 (1H, brs), 1.826-1.756 (2H, m), 1.499-1.427 (2H, m), 1.324-1.236 (14H, m), 0.904-0.869 (3H, m).
^{13}C NMR ($CDCl_3$): δ= 173.65, 156.97, 132.47, 129.98, 128.54, 127.65, 122.34, 120.14, 111.02, 76.77, 67.76, 65.81, 64.42, 61.69, 34.05, 31.94, 29.65, 29.61, 29.41, 29.37, 29.33, 26.26, 26.17, 22.71, 14.15.
HRMS (ESI-TOF [M+Na]$^+$) : Calcd. for $C_{26}H_{40}NaO_5Si^+$: 455.2768, Found 455.2758.
Anal. Calcd. for C, 72.19; H,9.32; N, 0.00. Found C, 72.10; H, 9.32; N, 0.00.

Compound **E-16**



Compound **E-13** (94.4 mg, 0.1642 mmol) was dissolved in THF (1 mL) and hydrogen fluoride-pyridine complex (97.0 μL) in pyridine (239.1 μL) was added to the solution above. This reaction mixture was stirred at room temperature under Ar atmosphere for 8 hrs. After 8 hrs, the reaction mixture was diluted with CH_2Cl_2 (10 mL) and water (10 mL) was added to the solution. Water layer was extracted with CH_2Cl_2 (10 mL×2), combined organic layer was washed with brine, dried over $MgSO_4$ and solvent was evaporated. The residue was purified by column chromatography (hexane : ethyl acetate = 2:1 to 1:1) to yield **E-16** (70.6 mg, 0.1533 mmol, 93.4 %, colorless oil).
^{1}H NMR ($CDCl_3$): δ= 7.377-7.326 (3H, m), 7.205-7.103 (4H, m), 7.079-7.022 (3H, m), 6.976-6.951 (1H, m), 6.920-6.861 (2H, m), 6.096-6.069 (1H, m), 5.946-5.896 (1H, m), 5.133-5.109 (1H, m), 5.061 (2H, s), 4.339-4.283 (1H, m), 4.222-4.153 (1H, m), 3.719-3.683 (1H, m), 3.642-3.595 (1H, m), 3.446-3.407 (1H, m), 3.049-2.936 (2H, m), 2.747-2.621 (2H, m), 2.300 (1H, brs).
^{13}C NMR ($CDCl_3$): δ= 173.55, 157.66, 156.92, 156.41, 139.28, 136.08, 132.54, 130.21, 129.97, 129.84, 129.55, 129.15, 128.80, 127.74, 124.90, 123.51, 122.29, 121.59, 120.84, 120.19, 119.20, 117.96, 117.11, 111.61, 111.06, 76.72, 69.31, 65.83, 65.34, 65.17, 64.47, 62.21, 61.70, 34.24, 34.03, 31.95, 29.67, 29.41, 26.22.
HRMS (ESI-TOF [M+Na]$^+$) : Calcd. for $C_{28}H_{28}NaO_6^+$: 483.1778, Found 483.1796.
Anal. Calcd. for C, 73.41; H,6.05; N, 0.00; (CH_2Cl_2×0.06). Found C, 72.13; H, 6.31; N, 0.00.

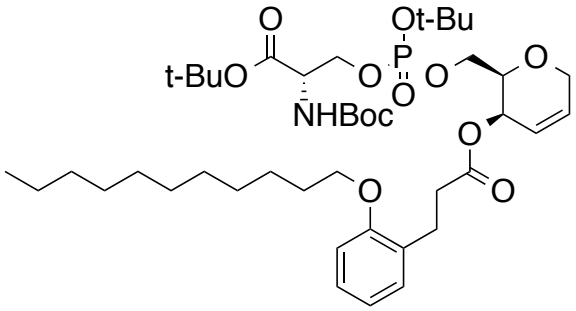
Compound **E-17**



Compound **A-3** (57.4 mg, 0.1271 mmol) and **E-14** (75.2 mg, 0.1906 mmol) was dissolved in CH_2Cl_2 (1 mL) and toluene (0.2 mL) and co-evaporated. The residue was dissolved in CH_2Cl_2 (1 mL) and *1H*-tetrazole (17.8 mg, 0.2541 mmol) in THF (1 mL) was added to the solution above. The whole was stirred at room temperature under Ar atmosphere for 16 hrs. After 16 hrs, the reaction mixture was diluted with CH_2Cl_2 (8 mL) and quenched by saturated $NaHCO_3$ aqueous solution (8 mL) and extracted with CH_2Cl_2 (8 mL×2). 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 to 2:1) to yield crude trivalent phosphodiester compound (79.6 mg, 0.1050 mmol, 55.1 %, colorless oil).

The trivalent phosphodiester compound (79.6 mg, 0.1050 mmol) was dissolved in CH_2Cl_2 (1 mL) *tert*-butylhydroperoxide in decane (0.0420 mL). The reaction mixture was stirred at room temperature under Ar atmosphere for 1.5hr. After 1.5hr, solvent was removed under vacuum and the residue was purified by column chromatography (hexane : ethyl acetate = 4:1 to 2:1) to yield **E-17** (74.4 mg, 0.0961 mmol, 91.6 %, colorless oil, 75.6 % (2 steps)).
^{1}H NMR ($CDCl_3$): δ= 6.032-5.998 (1H, m), 5.963-5.924 (1H, m), 5.503-5.448 (1H, m), 5.312-5.227 (2H, m), 5.024-5.002 (1H, m), 4.314-4.238 (3H, m), 4.178-3.956 (4H, m), 3.852-3.834 (1H, m), 2.280-2.242 (2H, m), 1.943-1.912 (4H, m), 1.563-1.527 (2H, m), 1.423-1.379 (27H, m), 1.226-1.196 (20H, m), 0.825-0.791 (3H, m).
^{13}C NMR ($CDCl_3$): δ= 173.18, 168.34, 155.29, 132.29, 132.24, 130.18, 129.96, 129.72, 122.19, 84.02, 83.94, 83.85, 82.59, 82.55, 79.86, 74.64, 74.57, 74.49, 67.58, 67.53, 66.20, 66.14, 66.00, 65.95, 65.59, 65.55, 65.55, 63.99, 63.90, 54.48, 54.39, 34.12, 34.09, 32.53, 31.88, 29.79, 29.75, 29.71, 29.68, 29.63, 29.50, 29.29, 29.15, 29.10, 29.08, 28.32, 27.94, 27.19, 27.16, 24.87, 24.83, 22.66, 14.09.
^{31}P NMR ($CDCl_3$): δ= -5.53, -5.82.
HRMS (ESI-TOF $[M+Na]^+$) : Calcd. for $C_{40}H_{72}NaO_{11}P^+$: 796.4741, Found 796.4714.
Anal. Calcd. for C, 62.04; H, 9.38; N, 1.81. Found C, 61.78; H, 8.98; N, 1.73.

Compound **E-18**

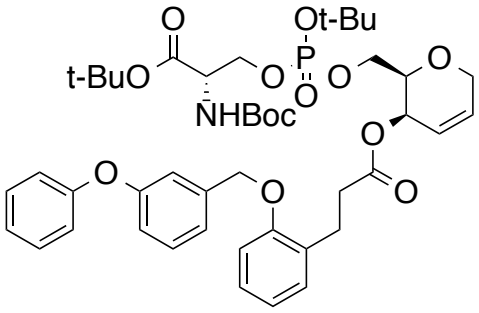


Compound **A-3** (85.6 mg, 0.1900 mmol) and **E-15** (123.0 mg, 0.2843 mmol) was dissolved in CH_2Cl_2 (1.5 mL) and toluene (0.2 mL) and co-evaporated. The residue was dissolved in CH_2Cl_2 (1.5 mL) and *1H*-tetrazole (26.6 mg, 0.3791 mmol) in THF (1.5 mL) was added to the solution above. The whole was stirred at room temperature under Ar atmosphere for 16 hrs. After 16 hrs, the reaction mixture was diluted with CH_2Cl_2 (10 mL) and quenched by saturated $NaHCO_3$ aqueous solution (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 (hexane : ethyl acetate = 4:1 to 1:1) to yield crude trivalent phosphodiester compound (111.2 mg, 0.1397 mmol, 73.53 %, colorless oil).

The trivalent phosphodiester compound (111.2 mg, 0.1397 mmol) was dissolved in CH_2Cl_2 (1 mL) *tert*-butylhydroperoxide in decane (0.0460 mL). The reaction mixture was stirred at room temperature under Ar atmosphere for 1.5 hr. After 1.5 hr, solvent was removed under vacuum and the residue was purified by column chromatography (hexane : ethyl acetate = 2:1 to 1:1) to yield **E-18** (102.3 mg, 0.1260 mmol, 90.2 %, colorless oil, 66.3 % (2 steps)).

^{1}H NMR ($CDCl_3$): δ= 7.157-7.105 (2H, m), 6.837-6.779 (2H, m), 6.061-6.027 (1H, m), 5.985-5.947 (1H, m), 5.555-5.512 (1H, m), 5.064-5.046 (1H, m), 4.362-4.321 (2H, m), 4.285-3.969 (5H, m), 3.944-3.895 (2H. m), 3.884-3.848 (1H, m), 2.977-2.846 (2H, m), 2.693-2.570 (2H, m), 1.805-1.735 (2H, m), 1.467-1.421 (28H, m), 1.342-1.217 (15H, m), 0.877-0.843 (3H, m).
^{13}C NMR ($CDCl_3$): δ= 172.80, 128.35, 156.92, 155.30, 132.19, 132.14, 129.92, 128.64, 127.52, 122.24, 120.11, 110.96, 84.00, 83.92, 83.84, 82.58, 82.56, 79.86, 74.69, 74.61, 74.60, 74.52, 67.74, 67.57, 67.52, 66.29, 66.24, 66.07, 65.56, 65.51, 64.16, 64.05, 54.49, 54.41, 33.91, 33.90, 31.90, 29.79, 29.75, 29,71, 29.62, 29.61, 29.58, 29.38, 29.33, 29.30, 28.32, 27.94, 26.11, 26.01, 22.67, 14.11.
^{31}P NMR ($CDCl_3$): δ= -5.50, -5.78.
HRMS (ESI-TOF [M+Na]$^+$) : Calcd. for $C_{42}H_{70}NNaO_{12}P^+$: 834.4528, Found 834.4535.
Anal. Calcd. for C, 60.70; H,8.44; N, 1.67; (CH_2Cl_2 ×0.3). Found C, 60.91; H, 8.15%; N, 1.74.

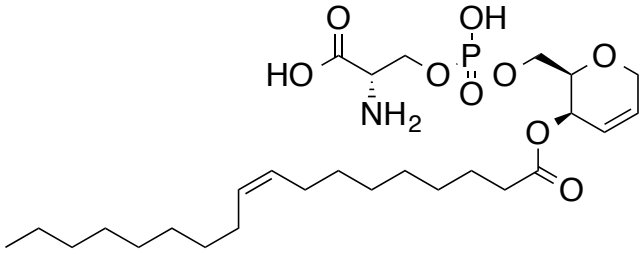
Compound **E-19**



Compound **A-3** (47.1 mg, 0.1044 mmol) and **E-16** (72.1 mg, 0.1566 mmol) was dissolved in CH_2Cl_2 (1 mL) and toluene (0.1 mL) and co-evaporated. The residue was dissolved in CH_2Cl_2 (1 mL) and *1H*-tetrazole (14.6 mg, 0.2087 mmol) in THF (1 mL) was added to the solution above. The whole was stirred at room temperature under Ar atmosphere for 21 hrs. After 21 hrs, the reaction mixture was diluted with CH_2Cl_2 (8 mL) and quenched by saturated $NaHCO_3$ aqueous solution (8 mL) and extracted with CH_2Cl_2 (8 mL×2). 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 to 2:1 to 1:1) to yield crude trivalent phosphodiester compound (53.9 mg, 0.0654 mmol, 62.7 %, colorless oil, **E-16** sm recovery : 37.2 mg (crude)).

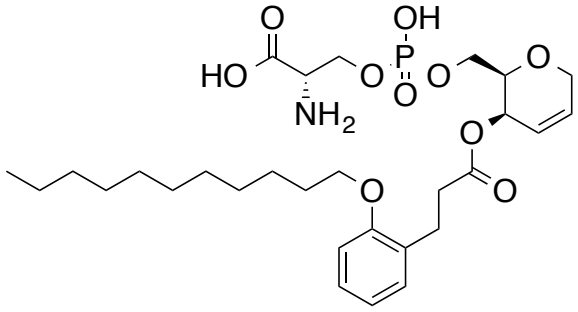
The trivalent phosphodiester compound (53.8 mg, 0.0653 mmol) was dissolved in CH_2Cl_2 (1 mL) tert-butylhydroperoxide in decane (0.0261 mL). The reaction mixture was stirred at room temperature under Ar atmosphere for 1.5 hr. After 1.5 hr, solvent was removed under vacuum and the residue was purified by column chromatography (hexane : ethyl acetate = 2:1 to 1:1) to yield **E-19** (53.8 mg, 0.0641 mmol, 98.1 %, colorless oil, 61.4 % (2 steps)).
^{1}H NMR ($CDCl_3$): δ= 7.357-7.306 (3H, m), 7.166-7.084 (4H, m), 7.035-6.993 (3H, m), 6.947-6.922 (1H, m), 6.892-6.827 (2H, m), 6.059-6.022 (1H, m), 5.977-5.942 (1H, m), 5.556-5.518 (1H, m), 5.055 (3H, m), 4.369-4.322 (2H, m), 4.286-4.266 (1H, m), 4.244-3.968 (4H, m), 3.899-3.852 (1H, m), 3.024-2.896 (2H, m), 2.703-2.582 (2H, m), 1.468-1.428 (27H, m).
^{13}C NMR ($CDCl_3$): δ= 172.68, 168.37, 157.62, 156.90, 156.35, 155.31, 139.33, 132.24, 132.18, 130.16, 129.96, 129.80, 128.91, 127.59, 123.46, 122.21, 121.52, 120.0, 119.17, 117.88, 117.03, 111.59, 83.95, 82.59, 79.89, 74.68, 74.60, 74.52, 69.29, 67.54, 66.28, 65.58, 65.52, 64.21, 64.10, 54.51, 33.88, 29.80, 29.76, 29.72, 28.33, 27.95, 25.97, 14.21.
^{31}P NMR ($CDCl_3$): δ= -5.46, -5.75.
HRMS (ESI-TOF [M+Na]$^+$) : Calcd. for $C_{44}H_{58}NNaO_{13}P^+$: 862.3538, Found 862.3549.
Anal. Calcd. for C, 58.97; H, 6.45; N, 1.53; (CH_2Cl_2×0.9). Found C, 58.92; H, 6.96; N, 1.62.

Compound **E-20**



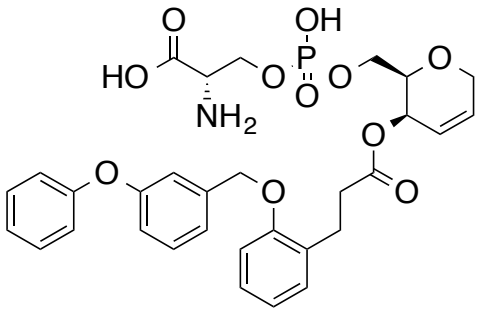
Compound **E-17** (74.3 mg, 0.0960 mmol) was dissolved in TFA (1.5 mL) at 0 °C and stirrted at 0 °C for 15 min and at room temperature for 1.5 hrs. The reaction mixture was evaporated and the residue was purified by column chromatography ($CHCl_3$: MeOH : H_2O = 65:25:4) to yield **E-20** (61.2 mg, 0.1090 mmol, 113.5 %, white solid (TFA salt)).
^{1}H NMR ($CDCl_3$): δ= 7.152-5.988 (3H, m), 5.410-5.303 (2H, m), 5.188-5.069 (1H, m), 4.580-4.402 (3H, m), 4.296-3.758 (4H, m), 2.448-2.361 (2H, m), 2.015-1.984 (2H, m), 1.645-1.496 (4H, m), 1.426-1.265 (20H, m), 0.885-0.852 (3H, m).
^{31}P NMR ($CDCl_3$): δ= -2.40.
HRMS (ESI-TOF [M-H]$^-$) : Calcd. for $C_{27}H_{47}NO_9P^-$: 560.2994, Found 560.2998.
Anal. Calcd. for C, 49.63; H, 6.86; N, 1.94; (CF_3CO_2H×1.4). Found C, 49.88; H, 6.89; N, 2.10.

Compound **E-21**



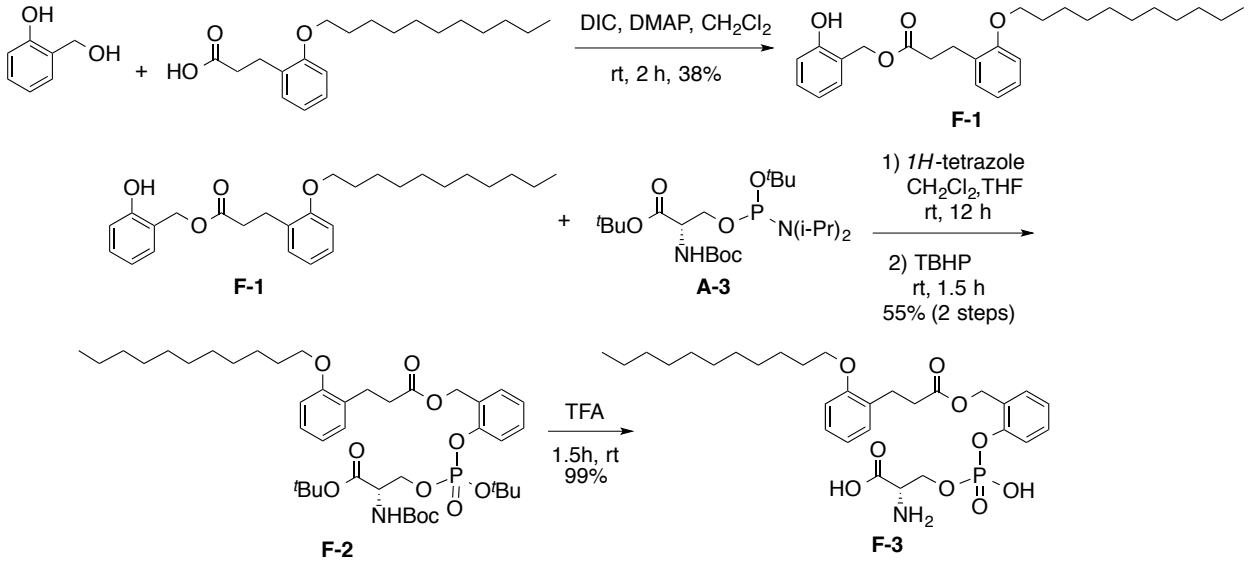
Compound **E-18** (92.9 mg, 0.1144 mmol) was dissolved in TFA (1.5 mL) at 0 °C and stirrted at 0 °C for 10 min and at room temperature for 1.5 hrs. The reaction mixture was evaporated and the residue was purified by column chromatography ($CHCl_3$: MeOH : H_2O = 65:25:4) to yield **E-21** (20.6 mg, 0.0344 mmol, 30.0 %, white solid).
^{1}H NMR ($CDCl_3$): δ= 7.203-7.116 (1H, m), 7.088-6.996 (1H, m), 6.877-6.813 (2H, m), 6.133-6.109 (1H, m), 5.904 (1H, m), 5.165 (1H, m), 4.590 (2H, m), 4.498-4.396 (2H, m), 4.291-4.248 (1H, m), 4.117(1H, m), 3.996-3.963 (4H, m), 2.941-2.860 (2H, m), 2.732 (2H, m), 1.791-1.711 (2H, m), 1.497-1.404 (2H, m), 1.297-1.264 (14H, m), 0.922-0.824 (3H, m).
^{31}P NMR ($CDCl_3$): δ= -2.07.
HRMS (ESI-TOF [M-H]$^-$) : Calcd. for $C_{29}H_{45}NO_{10}P^-$: 598.2787, Found 598.2862.
Anal. Calcd. for C, 46.15; H, 5.49; N, 1.58; (CF_3CO_2H×2.5). Found C, 45.81; H, 5.50; N, 1.76.

Compound **E-22**

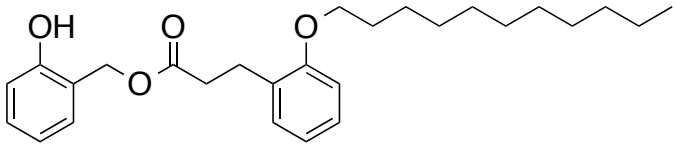


Compound **E-19** (50.5 mg, 0.0605 mmol) was dissolved in TFA (1.5 mL) at 0 °C and stirrted at 0 °C for 10 min and at room temperature for 2 hrs. The reaction mixture was evaporated and the residue was purified by column chromatography ($CHCl_3$: MeOH : H_2O = 65:25:4) to yield **E-22** (47.9 mg, 0.0763 mmol, 126.2 %, white solid (TFA salt)).
^{1}H NMR ($CDCl_3$): δ= 7.354-7.292 (3H, m), 7.195-7.109 (3H, m), 7.071-7.048 (2H, m), 7.010-6.990 (2H, m), 6.960-6.935 (1H, m), 6.899-6.871 (2H, m), 6.093-6.069 (1H, m), 5.850-5.838 (1H, m), 5.148 (1H, m), 5.067 (2H, s), 4.563 (1H, m), 4.455-4.365 (2H, m), 4.266-4.224 (1H, m), 4.095 (1H, m), 3.998-3.982 (2H, m), 3.009-2.882 (2H, m), 2.773-2.670 (2H, m).
^{31}P NMR ($CDCl_3$): δ= -2.29.
HRMS (ESI-TOF [M-H]$^-$) : Calcd. for $C_{31}H_{33}NO_{11}P^-$: 626.1797, Found 626.1809.
Anal. Calcd. for C, 42.32; H, 3.40; N, 1.24; (CF_3CO_2H×4.4). Found C, 42.37; H, 3.46; N, 1.58.

15. Synthesis of *ortho*-benzene-lysoPS analogues

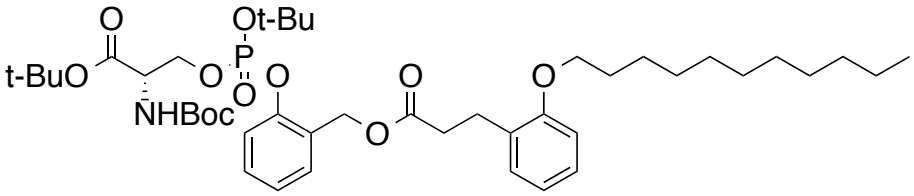


Compound **F-1**



DIC (204.2 mg, 1.6111 mmol) and DMAP (19.7 mg, 0.1611 mmol) were added to the solution of salicylic alcohol (100.0 mg, 0.8055 mmol) and acid derivative (258.2 mg, 0.8055 mmol) in CH_2Cl_2 (2 mL) and stirred at room temperature under Ar for 2 hrs. After 2 hrs, 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 MgSO4 and evaporated. The residue was purified by column chromatography (hexane : ethyl acetate = 20:1 (420 mL) to 10:1 (220 mL) to 4:1(250 mL)) to yield **F-1** (130.7 mg, 0.3064 mmol, 38.03 %, colorless oil).
^{1}H NMR ($CDCl_3$): δ= 7.357-7.283 (2H, m), 7.217 (1H, dt, *J*= 8.0 Hz, 7.6 Hz, 1.6 Hz), 7.127 (1H, dd, *J*= 7.6 Hz, 1.6 Hz), 7.011 (1H, dd, *J*= 8.0 Hz, 1.2 Hz), 6.963 (1H, dt, *J*= 7.6 Hz, 7.2 Hz, 1.2 Hz), 6.897-6.842 (2H, m), 4.001 (2H, t, *J*= 6.4 Hz), 3.025-2.987 (2H, m), 2.758-2.720 (2H, m) 1.882-1.812 (2H, m), 1.557-1.485 (2H, m), 1.387-1.355 (14H, m), 0.966 (3H, t, *J*= 6.8 Hz).
^{13}C NMR ($CDCl_3$): δ= 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, 76.02 ; H, 8.98; N, 0.00. Found C, 75.74; H, 8.82; N, 0.00.

Compound **F-2**

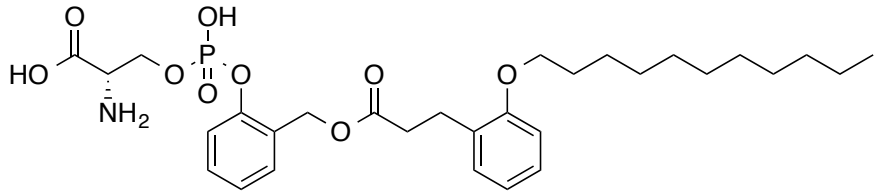


Compound **A-3** (177.4 mg, 0.3928 mmol) and **F-1** (128.9 mg, 0.3022 mmol) was dissolved in CH_2Cl_2 (2 mL) and toluene (0.2 mL) and co-evaporated. The residue was dissolved in CH_2Cl_2 (1 mL) and *1H*-tetrazole (63.5 mg, 0.9065 mmol) in THF (1 mL) was added to the solution above. The whole was stirred at room temperature under Ar atmosphere for 12 hrs. After 12 hrs, the reaction mixture was quenched by saturated $NaHCO_3$ aqueous solution (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 (hexane : ethyl acetate = 4:1 to yield crude trivalent phosphodiester compound (200.2 mg, 0.2534 mmol, 83.9 %, 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 hr. After 1.5 hr, solvent was removed under vacuum and the residue was purified by column chromatography (hexane : ethyl acetate = 4:1 (200 mL) to 2:1 (240 mL)) to yield **F-2** (132.7 mg, 0.1671 mmol, 66.0 %, colorless oil, 55.3 %(2 steps)).
^{1}H NMR ($CDCl_3$): δ= 7.306-7.169 (3H, m), 7.102-7.027 (3H, m), 6.767-6.726 (2H, m), 5.458-5.320 (1H, m), 5.144-5.135 (2H, m), 4.408-4.201 (3H, m), 3.886-3.853 (2H, m), 2.916-2.877 (2H, m), 2.633-2.594 (2H, m), 1.744-1.674 (2H, m), 1.433-1.416 (9H, m), 1.371-1.345 (19H, m), 1.255-1.155 (15H, m), 0.799 (3H, t, *J*= 6.8 Hz).
^{13}C NMR ($CDCl_3$): δ= 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,

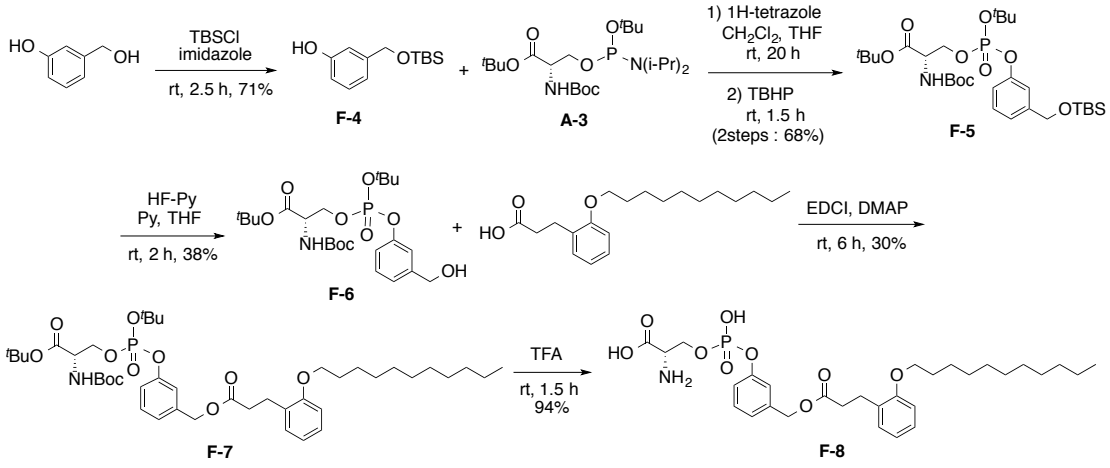
85.21, 82.77, 79 90, 77.41, 77.09, 76.77, 68.20, 68.14, 67.73, 60.90, 54.45, 54.39, 54.31, 34.12, 31.90, 29.79, 29.75, 29.61, 29.58, 29.36, 29.34, 29.32, 28.29, 27.92, 27.88, 26.16, 26.13, 22.67, 14.12.
^{31}P NMR ($CDCl_3$): δ= -11.03, -11.34.
HRMS (ESI-TOF [M+Na]$^+$): Calcd. for $C_{43}H_{68}NNaO_{11}P^+$: 828.4422 , Found 828.4421.
Anal. Calcd. for C, 63.58; H, 8.38; N, 1.72; (CH2Cl2×0.1). Found C, 63.80; H, 8.40; N, 1.64.

Compound **F-3**

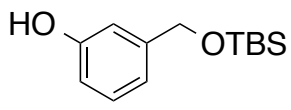


Compound **F-2** (127.0 mg, 0.1576 mmol) was dissolved in TFA (2 mL) at 0 °C and stirred at 0 °C for 10 min and at room temperature for 1.5 hr. The reaction mixture was evaporated and the residue was purified by column chromatography ($CHCl_3$: MeOH : AcOH = 6:1:2 to 6:1:3 to yield **F-3** (93.3 mg, 0.1572 mmol, 99.7 %, white solid,).
^{1}H NMR ($CDCl_3$): δ= 7.309-7.254 (2H, m), 7.226-7.120 (3H, m), 7.024-7.005 (1H, m), 6.892-6.824 (2H, m), 5.227 (2H, s), 4.690 (2H, m), 4.494 (1H, m), 3.991 (2H, t, *J*= 6.8 Hz), 2.972-2.935 (2H, m), 2.796-2.759 (2H, m), 1.820-1.749 (2H, m), 1.456-1.402 (2H, m), 1.322-1.253 (14H, m), 0.880 (3H, t, *J*= 6.8 Hz).
^{31}P NMR ($CDCl_3$): δ= -6.99.
HRMS (ESI-TOF [M-H]$^-$): Calcd. for $C_{30}H_{43}NO_9P^-$: 592.2681 , Found 592.2664.
Anal. Calcd. for C, 53.76; H, 6.27; N, 1.95; (CF3CO2H×1.1). Found C, 53.84; H, 6.29; N, 1.99.

16. Synthesis of *meta*-benzene lysoPS analogues

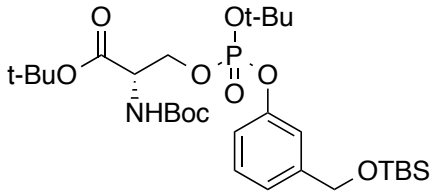


Compound **F-4**



m-Hydroxybenzylalcohol 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 acetate (10 mL×2), washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography (hexane : ethyl acetate = 2:1 to 1:1 to 1:2 to yield **F-4** (136.8 mg, 0.5738 mmol, 71.3 %).
^{1}H NMR ($CDCl_3$): δ= 7.067-7.028 (1H, m), 6.753-6.711(2H, m), 6.589-6.564 (1H, m), 5.836 (1H, brs), 4.584 (2H, s), 0.835 (9H, s), 0.000 (6H, s).
^{13}C NMR ($CDCl_3$): δ= 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, 65.05; H, 9.26; N, 0.00; (H_2O×0.1). Found C, 64.96; H, 9.03; N, 0.00.

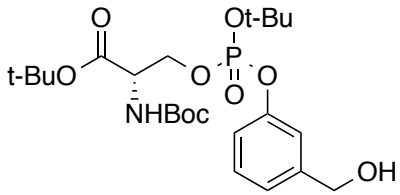
Compound **F-5**



Compound **A-3** (258.56 mg, 0.5726 mmol) and **F-4** (105.0 mg, 0.4404 mmol) was dissolved in CH_2Cl_2 (1 mL) and toluene (0.1 mL) and co-evaporated. 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 hrs. After 20 hrs, the reaction mixture was quenched by saturated $NaHCO_3$ aqueous solution (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 (hexane : ethyl acetate = 4:1) to yield crude trivalent phosphodiester compound (246.0 mg, 0.4088 mmol, 92.80 %, 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 hr. After 1.5 hr, solvent was removed under vacuum and the residue was purified by column chromatography (hexane : ethyl acetate = 4:1 to 2:1) to yield **F-5** (185.9 mg, 0.3009 mmol, 73.6 %, colorless oil, 68.3 % (2 steps)).
^{1}H NMR ($CDCl_3$): δ= 7.208-7.148 (1H, m), 7.054-7.014 (2H, m), 6.993-6.947 (1H, m), 5.388-5.291 (1H, m), 4.625 (2H, s), 4.384-4.322 (1H, m), 4.297-4.252 (1H, m), 4.244-4.196 (1H, m), 1.420-1.404 (9H, m), 1.350-1.334 (18H, m), 0.842 (9H, s), 0.000 (6H, s).
^{13}C NMR ($CDCl_3$): δ= 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 ($CDCl_3$): δ= -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, 56.38; H, 8.48; N, 2.27. Found C, 56.14; H, 8.35; N, 2.28.

Compound **F-6**



Compound **F-5** (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 hr. After 2 hr, 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 (hexane : ethyl acetate = 2:1 to 1:1 to 1:2 to yield **F-6** (57.7 mg, 0.1146 mmol, 38.2 %).

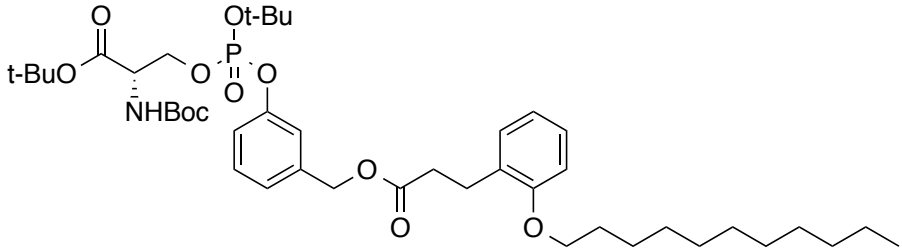
^{1}H NMR ($CDCl_3$): δ= 7.240-7.188 (1H, m), 7.148 (1H, m), 7.085-7.054 (1H, m), 7.024-6.978 (1H, m), 5.404-5.276 (1H, m), 4.587 (2H, s), 4.370-4.330 (1H, m), 4.331-4.233 (2H, m), 2.739 (1H, brs), 1.546-1.422 (9H, m), 1.372-1.322 (18H, m).

^{13}C NMR ($CDCl_3$): δ= 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 ($CDCl_3$): δ (ppm): δ = -11.30, -11.37.

HRMS (ESI-TOF [M+Na]$^+$): Calcd. for $C_{23}H_{38}NNaO_9P^+$: 526.2176, Found 526.2149.

Compound **F-7**



Compound **F-6** (57.6 mg, 0.1144 mmol) and carboxylic acid derivative (C3-ph-o-O-C11(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 mL×2), washed with brine, dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography (acetone : hexane = 1:5 / hexane : ethyl acetate = 2:1) to yield **F-7** (27.8 mg, 0.0346 mmol, 30.2% / sm recovery 6.2 mg, 15.4 %/ mixture 1.9 mg).

^{1}H NMR ($CDCl_3$): δ=7.247-7.206 (1H, m), 7.115-7.019 (5H, m), 6.783-6.736 (2H, m), 5.400-5.304 (1H, m), 5.013-5.008 (2H, m), 4.410-4.208 (3H, m), 3.894-3.861 (2H, m), 2.910-2.871 (2H, m), 2.628-2.590 (2H, m), 1.771-1.680 (2H, m), 1.449-1.433 (9H, m), 1.374-1.361 (20H, m), 1.308-1.191 (14H, m), 0.824-0.789 (3H, m).

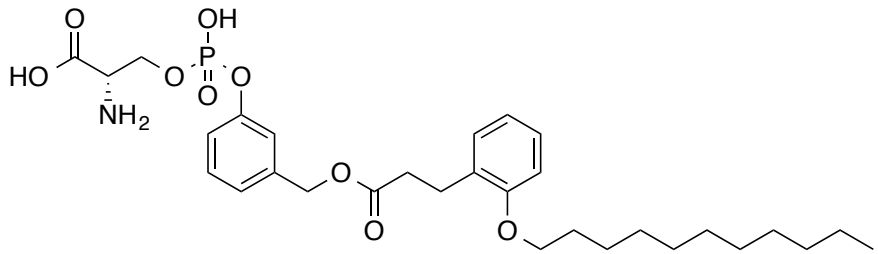
^{13}C NMR ($CDCl_3$): δ= 173.08, 168.17, 156.96, 155.20, 150.90, 150.84, 138.07, 129.95, 129.75, 128.75, 127.55, 124.37, 120.18, 119.70, 119.65, 119.60, 111.0085.10, 82.81, 79.99, 68.07, 67.97, 67.76, 65.36, 54.39, 34.11, 31.91, 29.81, 29.77, 29.62, 29.59, 29.36, 29.34, 29.31, 28.30, 27.93, 27.90, 26.16, 26.13, 25.61, 22.68, 14.12.

^{31}P NMR ($CDCl_3$): δ= -11.20, -11.50.

HRMS (ESI-TOF [M+Na]$^+$): Calcd. for $C_{43}H_{68}NNaO_{11}P^+$: 828.4422, Found 828.4431.

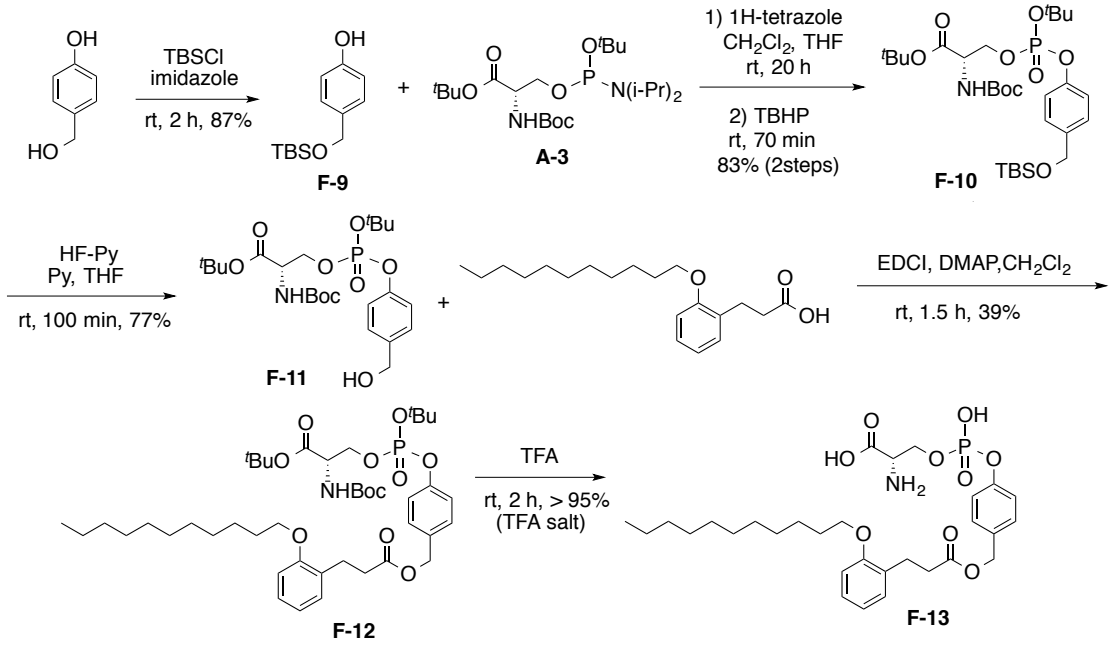
Anal. Calcd. for C, 64.08; H, 8.50; N, 1.74. Found C, 64.01; H, 8.44; N, 1.72.

Compound **F-8**

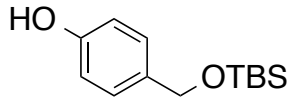


Compound **F-7** (23.5 mg, 0.0292 mmol) was dissolved in TFA (1.3 mL) at 0 °C and stirred at 0 °C for 10 min and at room temperature for 1.5 hr. The reaction mixture was evaporated and the residue was purified by column chromatography ($CHCl_3$: MeOH : H_2O= 65:25:4) to yield **F-8** (16.2 mg, 0.0273 mol, 93.5 %).
^{1}H NMR ($CDCl_3$): δ= 7.323-7.284 (1H, m), 7.201-7.158 (1H, m), 7.129-7.110 (1H, m), 7.051-7.026 (2H, m), 7.004-6.983 (1H, m), 6.870-6.807 (2H, m), 5.085 (2H, s), 4.643 (2H, m), 4.431 (1H, m), 3.994-3.961 (2H, m), 2.948-2.911 (2H, m), 2.755-2.718 (2H, m), 1.812-1.742 (2H, m), 1.467-1.398 (2H, m), 1.353-1.249 (14H, m), 0.889-0.854 (3H, m).
^{31}P NMR ($CDCl_3$): δ= -7.26.
HRMS (ESI-TOF [M-H]$^-$) : Calcd. for $C_{30}H_{43}NO_9P^-$: 592.2681, Found 592.2680.
Anal. Calcd. for C, 51.83; H, 5.96; N, 1.83; (CF_3CO_2H×1.5). Found C, 52.04; H, 6.09; N, 1.95.

17. Synthesis of *para*-benzene lysoPS analogue

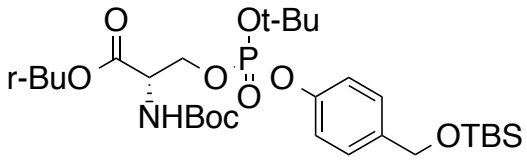


Compound **F-9**



Imidazole (258.8 mg, 3.8014 mmol) was added to the solution of 4-hydroxybenzyl alcohol (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 MgSO4 and evaporated under vacuum. The residue was purified by column chromatography (hexane : ethyl acetate = 4:1 to ethyl acetate) to yield **F-9** (357.0 mg, 1.4975 mmol, colorless oil, 86.7 %, sm recovery 7.9 mg).
^{1}H NMR ($CDCl_3$): δ= 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 ($CDCl_3$): δ= 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, 65.50; H,9.30; N, 0.00. Found C, 65.39; H, 9.25; N, 0.00.

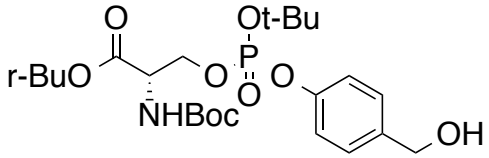
Compound **F-10**



Compound **A-3** (130.3 mg, 0.2885 mmol) and compound **F-9** (52.9 mg, 0.2219 mmol) was dissolved in CH_2Cl_2 (1 mL) and toluene (0.1 mL) and co-evaporated. 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 hrs. After 20 hrs, 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 (hexane : ethyl acetate = 4:1) to yield crude trivalent phosphodiester compound (141.8 mg, 0.2356 mmol, 106.2 %, colorless oil).

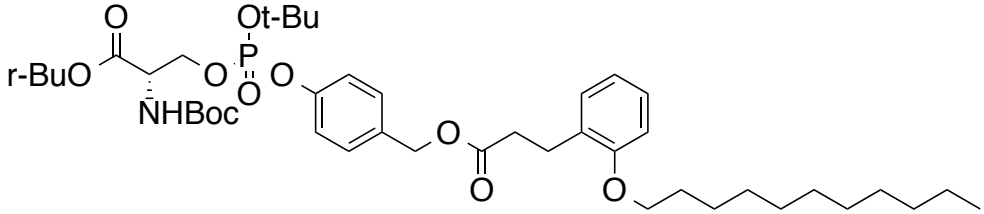
The trivalent phosphodiester compound (141.8 mg, 0.2356 mmol) was dissolved in CH_2Cl_2 (1.5 mL) and added *tert*-butylhydroperoxide in decane (0.0943 mL). The reaction mixture was stirred at room temperature under Ar atmosphere for 70 min. After 70 min, solvent was removed under vacuum and the residue was purified by column chromatography (hexane : ethyl acetate = 4:1 to 2:1) to yield **F-10** (114.2 mg, 0.1849 mmol, 78.46 %, white solid, 83.3 % (2 steps)).
^{1}H NMR ($CDCl_3$): δ= 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 ($CDCl_3$): δ= 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 ($CDCl_3$): δ= -11.04, -11.35.
HRMS (ESI-TOF $[M+Na]^+$): Calcd. for $C29H52NNaO_9PSi^+$: 640.3041, Found 640.3016.
Anal. Calcd. for C, 56.38; H, 8.48% N, 2.27. Found C, 56.14; H, 8.29; N, 2.22.

Compound **F-11**



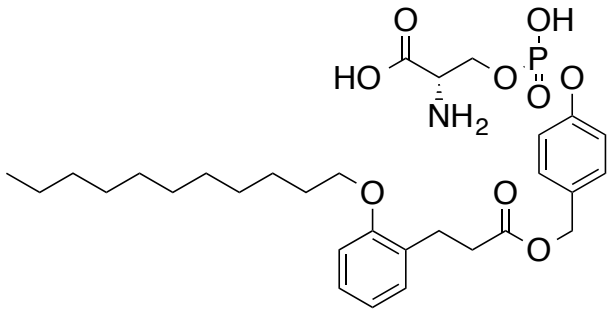
Compound **F-10** (107.2 mg, 0.1735 mmol) was dissolved in THF (1 mL) and hydrogen fluoride-pyridine complex (119.7 μL) in pyridine (294.7 μL) was added to the solution above. This reaction mixture was stirred at room temperature under Ar atmosphere for 100 min. After 100 min, the reaction mixture was quenched by water (10 mL). Water layer was extracted with CH_2Cl_2 (8 mL×2), combined organic layer was washed with brine, dried over $MgSO_4$ and solvent was evaporated. The residue was purified by column chromatography (hexane : ethyl acetate = 2:1 to 1:4) to yield **F-11** (67.3 mg, 0.1337 mmol, 77.04 %, colorless oil).
^{1}H NMR ($CDCl_3$): δ= 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 ($CDCl_3$): δ= 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 ($CDCl_3$): δ= -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, 54.86; H,7.61; N, 2.78; Found C, 54.91; H, 7.42; N, 2.84.

Compound **F-12**



Compound **F-11** (66.7 mg, 0.1325 mmol) and carboxylic acid (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 hrs. After 1.5hrs, 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 (hexane : ethyl acetate = 4:1 to 2:1) to yield **F-12** (42.1 mg, 0.0552 mmol, 39.4 %, colorless oil, 24.7 mg (mixture)).
^{1}H NMR ($CDCl_3$): δ= 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 ($CDCl_3$): δ= 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 ($CDCl_3$): δ= -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, 62.83; H, 8.50; N, 1.70. Found C, 62.93; H, 8.15; N, 1.73.

Compound **F-13**



Compound **F-12** (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 hr. The reaction mixture was evaporated and the residue was purified by column chromatography ($CHCl_3$: MeOH : H_2O= 65:25:4) to yield **F-13** (36.6 mg, 0.0607 mmol, 113.0 %, TFA salt).

^{1}H NMR ($CDCl_3$): δ= 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 ($CDCl_3$): δ= -6.77.

HRMS (ESI-TOF [M-H]$^-$): Calcd. for $C_{30}H_{43}NO_9P^-$: 592.2681, Found 592.2680.

Anal. Calcd. for C, 48.63; H, 5.76; N, 1.67 (TFA:X2, H_2OX1). Found C, 48.55; H, 5.86; N, 1.88.

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