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Article

Switching Lysophosphatidylserine G Protein-Coupled Receptor Agonists to Antagonists by Acylation of the Hydrophilic Serine Amine

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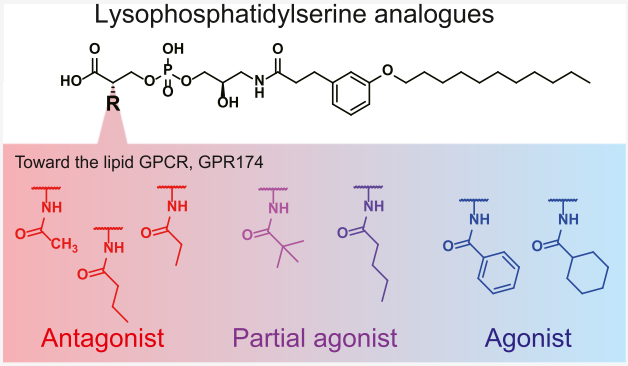
ABSTRACT: Three human G protein-coupled receptors (GPCRs)—GPR34/LPS_1, P2Y10/LPS_2, and GPR174/LPS_3—are activated specifically by lysophosphatidylserine (LysoPS), an endogenous hydrolysis product of a cell membrane component, phosphatidylserine (PS). LysoPS consists of L-serine, glycerol, and fatty acid moieties connected by phosphodiester and ester linkages. We previously generated potent and selective GPCR agonists by modification of the three modules and the ester linkage. Here, we show that a novel modification of the hydrophilic serine moiety, that is, N-acylations of the serine amine, converted a GPR174 agonist to potent GPR174 antagonists. Structural exploration of the amide functionality provided access to a range of activities from agonist to partial agonist to antagonist. The present study would provide a new strategy for the development of lysophospholipid receptor antagonists.



INTRODUCTION

Lysophospholipids (LPLs) are amphiphilic compounds that serve as signaling molecules (lipid mediators). Various LPLs are generated by enzymatic hydrolysis of an ester linkage of membrane phospholipids and are classified mainly by their hydrophilic moiety; for example, lysophosphatidic acid (LPA) has phosphoric acid as the hydrophilic head, while lysophosphatidylcholine (LPC) has phosphocholine. Among the LPLs, lysophosphatidylserine (LysoPS) is an emerging lysophospholipid mediator generated from phosphatidylserine (PS).

LysoPS is composed of three modules, L-serine, glycerol, and fatty acid, and two linkages, phosphodiester and ester, which connect the modules (Figure 1). It is distinct among LPLs in the fact that it has an amino acid, the serine moiety, as a hydrophilic head group. LysoPS specifically activates three G protein-coupled receptors (GPCRs) in humans: GPR34/LPS_1,[1] P2Y10/LPS_2,[2] and GPR174/LPS_3.[2] According to the BioGPS gene database (http://biogps.org/), these three receptors are highly expressed in several immune organs, including spleen and lymph nodes. GPR34 is also highly expressed in dorsal striatum. Studies using knock-out (KO) mice and single nucleotide polymorphism (SNP) analyses of these LysoPS receptors have revealed their roles in immunoregulation. For example, GPR34 activates the immune system to induce residence to *Cryptococcus neoformans* infection,[3] to produce pro-inflammatory cytokines,[3,4] and to promote phagocytosis[5] and cellular migration.[3,6] GPR174 is highly expressed in T cells and B cells[7,8] and negatively regulates their functions. An SNP of GPR174 is seen in autoimmune diseases such as Graves' disease[9−12] and Addison's disease,[13] and an altered expression of GPR174 is also associated with vasovagal syncope.[14] Recently, Shinjo et al. and later Barnes and Cyster showed that LysoPS acts via GPR174 to suppress interleukin-2 (IL-2) production in T cells.[8,15] Barnes et al. also showed that GPR174 is expressed on regulatory T cells (Tregs), and GPR174 signaling suppresses accumulation and activity of Tregs.[7] Indeed, GPR174 KO mice were less susceptible to the experimental autoimmune

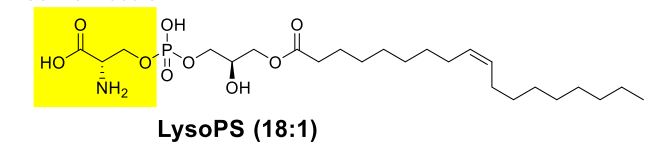


Figure 1. Structure of LysoPS (18:1).

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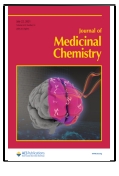

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encephalomyelitis than wild-type mice, and they suggested that antagonists of GPR174 might be promising therapeutics for autoimmune diseases.[7] Qiu et al. showed that GPR174 deficiency was protective against sepsis in mice by altering the levels of inflammatory agents, such as cytokines.[16] Thus, compounds that can modulate GPR174 activity, especially GPR174 antagonists, might be useful both in biological research and as candidate drugs for the control of autoimmune diseases, cancer, and chronic/acute inflammation. The function of P2Y10, compared to those of GPR34 and GPR174, is less elucidated.

Our group has focused on identifying specific agonists, antagonists, and inverse agonists that would be useful for elucidating the receptor functions, in addition to being potential starting points for drug discovery. Our previous structure−activity relationship (SAR) studies showed that these LysoPS receptors strictly recognize the serine moiety.[17,18] Some modification of the serine moiety is tolerated by GPR174 but less so by GPR34 or P2Y10. For example, LysoPalloT, in which the serine moiety is replaced with *allo*-threonine, shows diminished activity for the activation of GPR34 and P2Y10, but retains activity toward GPR174, and thus shows increased subtype selectivity for GPR174.[18] The carboxylic acid functionality of the serine moiety was shown to be essential for the activation of all LysoPS receptors because methyl esterification and elimination of the carboxylic acid functionality to afford a lysophosphatidylethanolamine derivative, which is also an endogenous LPL, resulted in a marked decrease or loss of activity toward all the LysoPS receptors.[18] However, the structure requirements for the hydrophilic module, especially regarding modification of the amino group, are not yet fully understood.

In this study, we aimed to elucidate the effect of the amino acid moiety, particularly the amino group, on the activities of LysoPS analogues toward GPR174. Notably, our results indicate that structural modifications at this location can switch the activity of these analogues from agonistic to partial agonistic to antagonistic.

RESULTS AND DISCUSSION

Molecular Design for Structural Change of the Hydrophilic Moiety on GPR174 Agonists. Since GPR174 is the least susceptible to modification of the hydrophilic serine part of the prototype LysoPS among the three LysoPS receptors, we focused on the effect of structural change of the hydrophilic moiety on GPR174 activation. We previously identified some structural requirements favorable for GPR174 activation:[18] (1) conversion of the ester linkage between the glycerol and the fatty acid moiety to an amide bond and (2) use of the *meta*-substituted benzene system as a non-fatty acid surrogate. Thus, we studied the SAR of analogues containing these two modifications (Figure 2).

The synthetic schemes of the LysoPS analogues studied here are shown in Scheme 1 and in Experimental Section. Functional groups were protected with benzyl groups and removed by hydrogenation in the final step. Since all the LysoPS analogues studied here contain the original 2*R*-glycerol moiety, we synthesized compound **2**, which is a common building block of the fatty acid and glycerol moiety of LysoPS analogues, in four steps from the benzene-containing fatty acid surrogate **1**[18] as described previously.[6] The phosphodiester linkage between compound **2** and various hydrophilic head moieties (general structure **I** in Scheme 1) was generated with benzyl *N*,*N*,*N*′,*N*′-

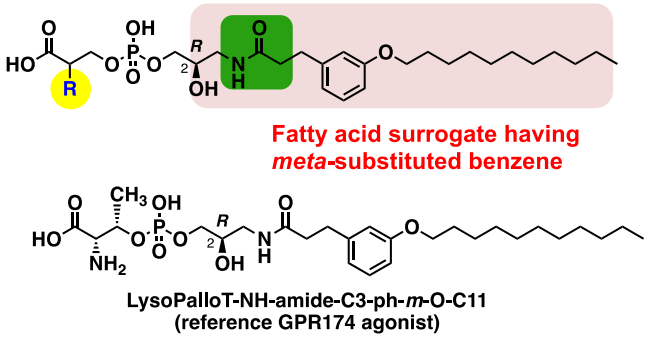

Figure 2. Molecular design of GPR174 antagonists.

tetraisopropylphosphorodiamidite (compound **3**) using the phosphoramidite method. The detailed synthetic route of each hydrophilic head moiety (structure **I**) is described in Experimental Section. We employed the procedure reported by Aurelio et al.[19] for the synthesis of the hydrophilic head of **7c** (Table 1), in which the serine amine is mono-methylated.

Two routes (**A** and **B** in Scheme 1), which differ in the order of P−O bond formation in the coupling with hydrophilic head moiety **I** and compound **2**, are possible, and we chose one of these two routes by considering the feasibility of product purification. Thus, in route A, compound **3** is subjected to react first with alcohol **2** to yield compound **4**, followed by coupling with the hydrophilic head moiety **I** (Scheme 1), or in route B, the hydrophilic head moiety **I** first reacts with **3** to yield intermediate **II**, and then **II** is coupled with **2** to afford **III** (**II** and **III** are general structures with a various R group, Scheme 1). Compound **III** was finally deprotected by hydrogenation to yield the target LysoPS analogue (general structure **IV**). We showed the synthesis of the derivative **7d** through route A as a representative example in Scheme 1: the hydrophilic head part **5** was synthesized by acylation of the amino group in L-serine benzyl ester hydrochloride. Compound **5** was coupled with phosphoramidite **4** to yield compound **6**. Global deprotection of **6** by means of hydrogenation in THF afforded the final product **7d**. In this hydrogenation process, THF was a better solvent than conventional alcohol such as methanol because methanol generated the methyl ester of the final carboxylic acid product, probably due to increased intrinsic acidity of the carboxylic acid of the target LysoPS analogue, due to the lack of the basic amino group.

Discovery of a GPR174 Antagonist by α-Substitution of the Serine Moiety. We evaluated the activities of the α-substituted serine analogues by means of the previously described transforming growth factor-α (TGFα) shedding assay (Tables 1−4 and Figures 3, 4, and S1−S3).[2] In this assay, the GPCR activation level is evaluated as the level of the ectodomain shedding of a membrane-bound preform of alkaline phosphatase (AP)-tagged TGFα (AP-TGFα). Taking into account the use of mice in future *in vivo* studies, mouse GPR174 was used for the evaluation of LysoPS analogues. LysoPS analogues evaluated in this study showed almost no agonistic activity or very low activity toward mouse GPR34 or P2Y10, as compared with endogenous pan-agonist LysoPS (18:1) (data not shown), and we focus here on the activity toward mouse GPR174.

Compound **7a**, which has the (*S*)-amino group like endogenous LysoPS, was a highly active and subtype-selective agonist toward GPR174, as expected (EC_{50}: 1.6 nM) (Table 1 and Figures 3a and S2a). However, **7a** showed a slight reduction

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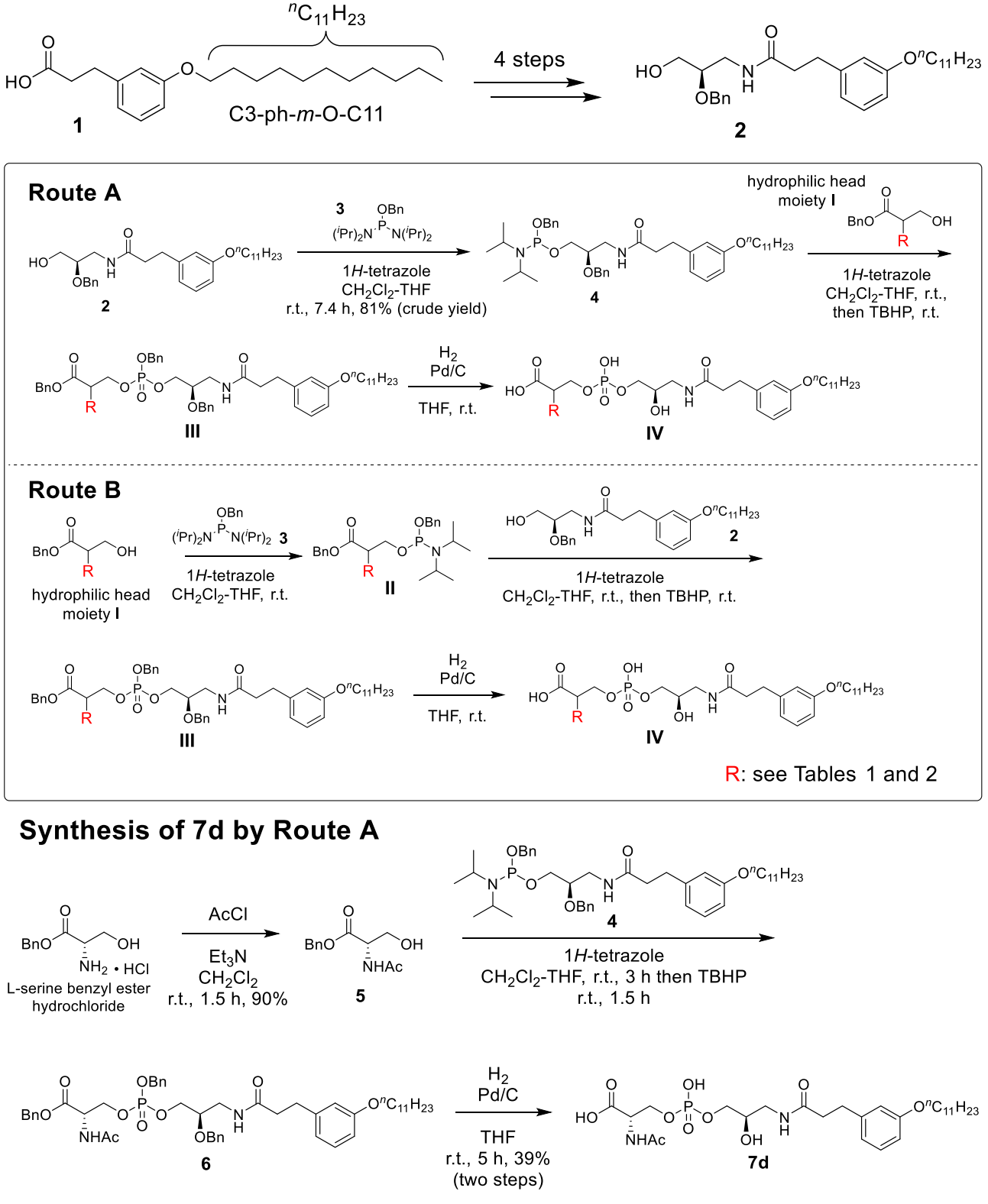
Scheme 1. Synthesis of LysoPS Analogues



in E_{max}, the maximum TGFα shedding response (defined in Figure 3) (E_{max} = 71.7 ± 5.4%) compared with LysoPS (18:1) (E_{max} = 100 ± 0%) (Table 1). Next, we introduced an (*S*)-methyl group (**7b**) instead of the amino group as the R group. Methyl-substituted **7b** showed partial agonistic activity with greatly reduced E_{max} (EC_{50}: 380 nM and E_{max} = 29.9 ± 7.1%) toward GPR174. The analogues with a mono-methylated (**7c**) or acetylated (**7d**) (*S*)-amino group showed impaired or no agonistic activity (**7c** EC_{50}: 51 nM, E_{max} = 20.1 ± 2.1%; **7d** EC_{50} ND). Instead, **7d** inhibited the GPR174 activation level to below the basal state (Figures 3a and S2a), and thus, **7d** showed inverse agonistic activity toward GPR174.

We also evaluated the effect of stereochemistry of α-amino and α-methyl substituents on GPR174 agonistic ability (Table 1

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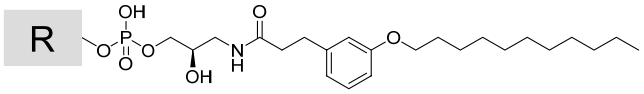
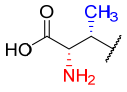
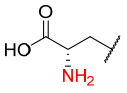
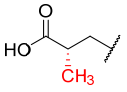
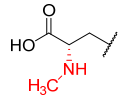
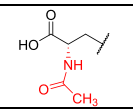
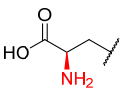
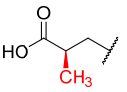
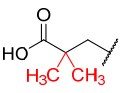
Table 1. Structure Expansion of α-Substitution of the Serine Moiety of the GPR174 Agonist[a]

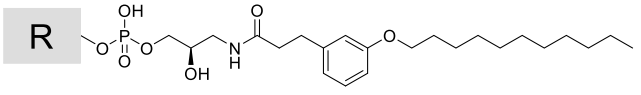


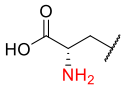
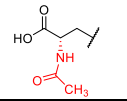
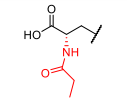
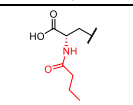
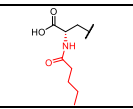
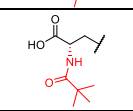
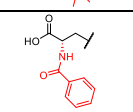
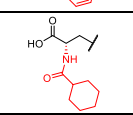
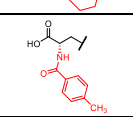
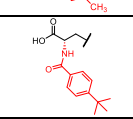
	R	GPR174	
		EC_{50} (pEC_{50}) [E_{max}] n =	IC_{50} (pIC_{50}) [I_{max}] n =
Positive Control		34 nM (7.47 ± 0.11) [87.7 ± 7.7%] 12	
18:1-LysoPS		34 nM (7.47 ± 0.09) [100 ± 0%] 29	
7a		1.6 nM (8.80 ± 0.11) [71.7 ± 5.4%] 6	
7b		380 nM (6.42 ± 0.23) [29.9 ± 7.1%] 3	4.7 nM (8.32 ± 0.17) [69.5 ± 8.9%] 3
7c		51 nM (7.29 ± 0.08) [20.1 ± 2.1%] 3	ND (ND) [ND] 3
7d		ND (ND) [ND] 3	5.6 nM (8.25 ± 0.03) [117.7 ± 6.4%] 3
7e		81 nM (7.09 ± 0.19) [70.9 ± 18.2%] 3	
7f		0.9 nM (9.05 ± 0.15) [28.5 ± 3.2%] 3	12.5 nM (7.90 ± 0.06) [59.7 ± 4.5%] 3
7g		> 3 μM (< 5.5) [NA] 4	1100 nM (5.94 ± 0.07) [106.8 ± 7.8%] 3

[a]Activities are represented in terms of EC_{50}, IC_{50}, pEC_{50}, pIC_{50}, E_{max}, or I_{max}: pEC_{50} and pIC_{50} are calculated from the sigmoidal concentration−response curve and shown as mean ± SEM (standard error of the mean) of the indicated numbers of independent experiments (n); EC_{50} and IC_{50} values are calculated from the mean value of pEC_{50} and pIC_{50}, respectively; the E_{max} values of the analogues are calculated as the percentage of the maximum response obtained by LysoPS (18:1) (see Figure 3, frame); I_{max} values are calculated as the percentage of the span of the sigmoidal concentration−activity response curves divided by the maximum response in the absence of the inhibitor (see Figure 4, frame). "NA" means "not available" because of very low activity and "ND" means "not determined" because of no agonistic or antagonistic activity.

and Figures 3a and S2a). (*R*)-α-Amino analogue **7e** showed diminished agonistic activity, with only about one-fiftieth of the potency of (*S*)-α-amino **7a** (**7e** EC_{50} = 81 nM and **7a** EC_{50} = 1.6 nM). The (*R*)-α-methyl analogue **7f** showed partial agonistic activity toward GPR174 (EC_{50} 0.9 nM, E_{max} = 28.5 ± 3.2%). The α,α-dimethyl analogue **7g** also showed almost no agonistic potency toward GPR174 (EC_{50} > 3 μM) (Table 1 and Figures 3a and S2a).

Table 2. Exploration of the Effect of Amide Substituents on Receptor Activation[a]



	R	GPR174	
		EC_{50} (pEC_{50}) [E_{max}] n =	IC_{50} (pIC_{50}) [I_{max}] n =
7a		1.6 nM (8.80 ± 0.11) [71.7 ± 5.4%] 6	
7d		ND (ND) [ND] 3	5.6 nM (8.25 ± 0.03) [117.7 ± 6.4%] 3
8a		> 3 μM (< 5.5) [NA] 3	3.4 nM (8.47 ± 0.15) [103.2 ± 1.6%] 3
8b		> 3 μM (< 5.5) [NA] 3	5.4 nM (8.26 ± 0.06) [111.2 ± 4%] 3
8c		0.58 nM (9.23 ± 0.22) [38.7 ± 2.2%] 3	6.7 nM (8.18 ± 0.05) [41 ± 0%] 3
8d		1.1 nM (8.96 ± 0.02) [20 ± 4.6%] 4	3.8 nM (8.42 ± 0.17) [71.6 ± 9.7%] 3
8e		13 nM (7.87 ± 0.07) [52.1 ± 9.2%] 4	ND (ND) [ND] 3
8f		22 nM (7.66 ± 0.07) [69.5 ± 1.8%] 3	ND (ND) [ND] 3
8g		9 nM (8.02 ± 0.21) [31.6 ± 3%] 4	190 nM (6.73 ± 0.47) [86.2 ± 12.7%] 3
8h		ND (ND) [ND] 3	700 nM (6.15 ± 0.12) [96.1 ± 11.8%] 3

[a]Activities are represented in terms of EC_{50}, IC_{50}, pEC_{50}, pIC_{50}, E_{max}, or I_{max}: pEC_{50} and pIC_{50} are calculated from the sigmoidal concentration−response curve and shown as mean ± SEM of the indicated numbers of independent experiments (n); the EC_{50} and IC_{50} values are calculated from the mean value of pEC_{50} and pIC_{50}, respectively; the E_{max} values of the analogues are calculated as the percentage of the maximum response obtained by LysoPS (18:1) (see Figure 3, frame); the I_{max} values are calculated as the percentage of the span of the sigmoidal concentration−activity response curves divided by the maximum response in the absence of the inhibitor (see Figure 4, frame). "NA" means "not available" because of very low activity and "ND" means "not determined" because of no agonistic or antagonistic activity.

As small modifications of the substituent at the α-position dramatically changed the GPR174 agonistic potency, we

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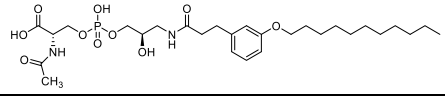
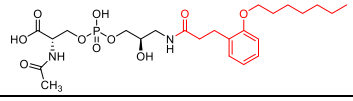
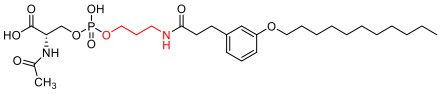
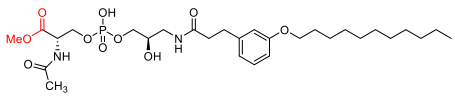
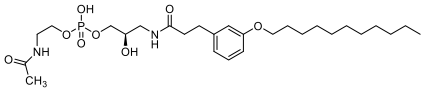
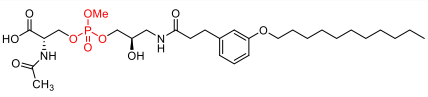
Table 3. Change of Antagonistic Activities upon Structural Perturbation[a]

		GPR174	
		EC_{50} (pEC_{50}) [E_{max}] n =	IC_{50} (pIC_{50}) [I_{max}] n =
7d		ND (ND) [ND] 3	5.6 nM (8.25 ± 0.03) [117.7 ± 6.4%] 3
9a		ND (ND) [ND] 4	> 3 μM (5.50 ± 0.10) [NA] 3
9b		ND (ND) [ND] 3	270 nM (6.58 ± 0.26) [80.6 ± 12.2%] 3
9c		ND (ND) [ND] 3	610 nM (6.22 ± 0.06) [103.3 ± 3.6%] 3
9d		> 3 μM (< 5.5) [NA] 3	ND (ND) [ND] 3
9e		ND (ND) [ND] 3	51 nM (7.29 ± 0.11) [108.6 ± 11.7%] 3

[a]Activities are represented in terms of EC_{50}, IC_{50}, pEC_{50}, pIC_{50}, E_{max}, or I_{max}: pEC_{50} and pIC_{50} are calculated from the sigmoidal concentration−response curve and shown as mean ± SEM of the indicated numbers of independent experiments (n); EC_{50} and IC_{50} values are calculated from the mean value of pEC_{50} and pIC_{50}, respectively; the E_{max} values of the analogues are calculated as the percentage of the maximum response obtained by LysoPS (18:1) (see Figure 3, frame); the I_{max} values are calculated as the percentage of the span of the sigmoidal concentration−activity response curves divided by the maximum response in the absence of the inhibitor (see Figure 4, frame). "NA" means "not available" because of very low activity and "ND" means "not determined" because of no agonistic or antagonistic activity.

suspected that these changes altered the efficacy, not the affinity, of the analogues for the receptor. Thus, we evaluated the inhibitory activities of these analogues in the presence of a previously reported GPR174 agonist, **LysoPalloT-NH-amide-C3-ph-*m*-O-C11**[18] (Table 1 and Figures 4a and S3a). The results of TGFα shedding assay of selected analogues showed that mono-α-methyl analogues, **7b** and **7f**, have moderate inhibitory activity, whereas α,α-dimethyl analogue **7g** has decreased inhibitory activity: **7b** IC_{50} = 4.7 nM; **7f** IC_{50} = 12.5 nM; and **7g** IC_{50} = 1100 nM. Compounds **7b** and **7f**, which have partial agonistic activity toward GPR174, did not completely inhibit the activation of GPR174 by the positive agonist even at their maximum concentration (Figures 4a and S3a). We define I_{max} as the percentage of the activation decrease by an inhibitor with respect to the span of the activation by the positive agonist (Figure 4). When the analogue completely inhibits the activation by the positive agonist, its I_{max} is 100%. The I_{max} values of **7b** and **7f** were 69.5 ± 8.9 and 59.7 ± 4.5%, respectively (Table 1). The N-methylated analogue **7c** showed almost no antagonistic activity, but the N-acetylated analogue **7d** potently inhibited the activation by **LysoPalloT-NH-amide-C3-ph-*m*-O-C11** (**7c** IC_{50} ND; **7d** IC_{50} = 5.6 nM, I_{max} = 117.7 ± 6.4%) (Table 1 and Figures 4a and S3a). Thus, simple N-acetylation of the serine amino group of the GPR174 agonist **7a** affords the GPR174 antagonist **7d**.

Extension of the α-Amide Moiety Switches Activity toward GPR174 Antagonism. As the α-amide analogue **7d** showed potent inhibitory activity toward GPR174, we next focused on the *N*-acyl (amide) group of the hydrophilic serine amine and explored amide analogues (Table 2 and Figures 3b, 4b, S2b, and S3b).

We elongated the alkyl chain at the α-serine amide of **7d** (C2) to obtain **8a** (C3), **8b** (C4), and **8c** (C5) (in parentheses: number of carbon atom in the acyl group, Table 2). Compounds **8a** (C3) and **8b** (C4) showed GPR174 antagonistic activity as potent as that of **7d** (C2) (**7d** IC_{50} = 5.6 nM; **8a** IC_{50} = 3.4 nM; and **8b** IC_{50} = 5.4 nM; Table 2 and Figures 4b and S3b). However, **8c** (C5), which bears a pentanamide, showed partial agonistic activity toward GPR174 (EC_{50} = 0.58 nM and E_{max} = 38.7 ± 2.2%) compared with the agonist **7a**; the E_{max} value of **8c** was lower than that of the agonist **7a** (**7a** E_{max} = 71.7 ± 5.4% and **8c** E_{max} = 38.7 ± 2.2%, Table 2 and Figures 3b and S2b). Also, the maximum inhibition by **8c** was weaker than that by the full antagonist **7d** (**7d** I_{max} = 117.7 ± 6.4% and **8c** I_{max} = 41 ± 0%) (Table 2 and Figures 4b and S3b).

The pivalamide analogue **8d** (C5) also showed partial agonistic activity toward GPR174 (EC_{50} = 1.1 nM), but its E_{max} value was lower than that of **8c**; that is, **8d** has a more antagonistic characteristic than the partial agonist **8c** (**8c** E_{max} = 38.7 ± 2.2% and **8d** E_{max} = 20 ± 4.6%, Table 2 and Figures 3b and S2b). While the molecular weights of **8c** and **8d** are the same, these different behaviors of **8c** and **8d** suggest that a more extended amide structure is likely to favor the agonistic activity. Therefore, we synthesized *N*-benzoyl derivatives (**8e−8h**). Benzamide analogue **8e** and non-aromatic cyclohexanecarboxyamide analogue **8f** showed agonistic activity with E_{max} as high as that of **7a** (**7a** E_{max} = 71.7 ± 5.4%; **8e** E_{max} = 52.1 ± 9.2%; and **8f** E_{max} = 69.5 ± 1.8%, Table 2 and Figures 3b and S2b), and no

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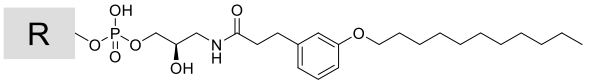
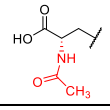
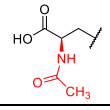
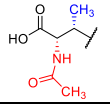
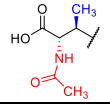
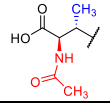
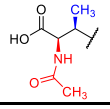
Table 4. Effect of Stereochemistry of the *N*-Acetyl Group and *β*-Methylation[a]



	R	GPR174	
		EC_{50} (pEC_{50}) [E_{max}] n =	IC_{50} (pIC_{50}) [I_{max}] n =
7d		ND (ND) [ND] 3	5.6 nM (8.25 ± 0.03) [117.7 ± 6.4%] 3
10a		ND (ND) [ND] 3	360 nM (6.44 ± 0.10) [95.2 ± 16.8%] 3
10b		ND (ND) [ND] 3	100 nM (6.99 ± 0.03) [106.1 ± 9.4%] 3
10c		ND (ND) [ND] 3	120 nM (6.91 ± 0.07) [103 ± 5.7%] 3
10d		ND (ND) [ND] 3	ND (ND) [ND] 3
10e		ND (ND) [ND] 3	ND (ND) [ND] 3

[a]Activities are represented in terms of EC_{50}, IC_{50}, pEC_{50}, pIC_{50}, E_{max}, or I_{max}: pEC_{50} and pIC_{50} are calculated from the sigmoidal concentration−response curve and shown as mean ± SEM of the indicated numbers of independent experiments (n); the EC_{50} and IC_{50} values are calculated from the mean value of pEC_{50} and pIC_{50}, respectively; the E_{max} values of the analogues are calculated as the percentage of the maximum response obtained by LysoPS (18:1) (see Figure 3, frame); the I_{max} values are calculated as the percentage of the span of the sigmoidal concentration−activity response curves divided by the maximum response in the absence of the inhibitor (see Figure 4, frame). "NA" means "not available" because of very low activity and "ND" means "not determined" because of no agonistic or antagonistic activity.

antagonistic activity was detected in the presence of agonist **LysoPalloT-NH-amide-C3-ph-*m*-O-C11** (Table 2 and Figures 4b and S3b). Compound **8g**, which has a *p*-methyl group on the benzamide moiety of **8e**, showed partial agonistic activity (E_{max} = 31.6 ± 3%) (Table 2 and Figures 3b and S2b). The agonistic activity of **8g** was lower than that of **8c** in terms of EC_{50} (**8c** EC_{50} = 0.58 nM and **8g** EC_{50} = 9 nM). Compound **8h**, which has a *p*-*t*-butyl substituent on the benzamide, was inactive toward GPR174 in the agonist assay and its inhibitory activity was greatly diminished compared to that of **7d** (**8h** EC_{50} ND, IC_{50} = 700 nM) (Table 2 and Figures 3b, 4b, S2b, and S3b). As a substituent on the benzamide moiety decreases the agonistic activity, this region of **8e** may be involved in recognition by the receptor GPR174. Thus, the agonist, partial agonist, or antagonist characteristic of these analogues appears to depend on the structure of the *α*-amide moiety in the hydrophilic structure. These results suggest that a linearly extended amide is favorable for the GPR174 agonistic activity and that the hydrophobic space around *N*-alkyl amide may be limited.

Change of Antagonistic Activities upon Structural Perturbation. With potent GPR174 antagonists such as **7d** in hand, we next focused on the structural requirements for antagonists. In our previous SAR studies, we had identified some important structural elements that increase the GPR174 *agonistic* activity: the structure of the fatty acid part and the presence of a hydroxyl group at the glycerol moiety and a carboxyl acid group at the serine moiety.[18] We examined the change of biological activities of antagonist **7d** in response to changes of the fatty acid surrogate, elimination of the glycerol hydroxyl group, methyl esterification, and O-methylation of the phosphodiester (Table 3 and Figures 3c, 4c, S2c, and S3c). The results indicate that the structural requirements for GPR174 antagonists and agonists are similar.

The agonistic activities of LysoPS analogues toward GPR174 are strongly influenced by the structure of the hydrophobic fatty acid moiety,[18,20] and the introduction of a fatty acid surrogate having an *ortho*-substituted benzene ring and short alkoxy chain (OC_7H_{15}) (named as fatty acid *o*-O-C7 here) dramatically decreased the agonistic activity.[18] When *o*-O-C7 was introduced into the GPR174 antagonist **7d** instead of the *meta*-substituted fatty acid surrogate, the analogue **9a** showed almost no antagonistic activity toward GPR174 (IC_{50} > 3 μM) (Table 3 and Figures 4c and S3c). Thus, the fatty acid moiety of the GPR174 antagonist **7d** is recognized by the receptor, probably in a similar manner to that of GPR174 agonists.

A hydroxyl group on the glycerol moiety is also important for the GPR174 agonistic activity.[18,20] Deletion of the glycerol hydroxyl group in antagonist **7d** dramatically reduced its antagonistic activity (**9b** IC_{50} = 270 nM) (Table 3 and Figures 4c and S3c). Thus, the glycerol hydroxyl group should play an important role in the interaction with GPR174 in the antagonist-bound form, as in the case of agonists.

When the carboxyl group of antagonist **7d** was converted to methyl ester (**9c**), the IC_{50} value for antagonistic activity was decreased dramatically (**7d** IC_{50} = 5.6 nM and **9c** IC_{50} = 610 nM) (Table 3 and Figures 4c and S3c). Deletion of the carboxyl group in **7d** also abolished the GPR174 antagonistic activity (**9d** IC_{50} ND) (Table 3 and Figures 4c and S3c). On the other hand, when the phosphodiester of **7d** was O-methylated (**9e**), the antagonistic activity was still about one-ninth of that of **7d** (**7d** IC_{50} = 5.6 nM and **9e** IC_{50} = 51 nM) (Table 3 and Figures 4c and S3c). Thus, although both the carboxylate and the phosphodiester of the GPR174 antagonist **7d** are recognized by the receptor, the carboxylic acid functionality is more important for the antagonist−receptor interaction. A similar trend was previously observed for GPR174 agonists: methylation of the carboxylic acid group or methylation of the phosphodiester reduced the GPR174 agonistic activity.[18] Thus, structural perturbations such as changes of the fatty acid surrogate, elimination of the glycerol hydroxyl group, methyl esterification, and O-methylation of phosphodiester have similar effects on the biological activities of antagonist **7d** and agonists.[18] These results are consistent with the idea that the structural requirements for the activity of both GPR174 agonists and antagonists are similar.

Effect of Stereochemistry of the N-Acetyl Group and *β*-Methylation. Conversion of (*S*)-acetamide stereochemistry of **7d** to (*R*)-acetamide greatly decreased the GPR174 antagonistic activity (**10a** IC_{50} = 360 nM, Table 4 and Figures 4d and S3d).

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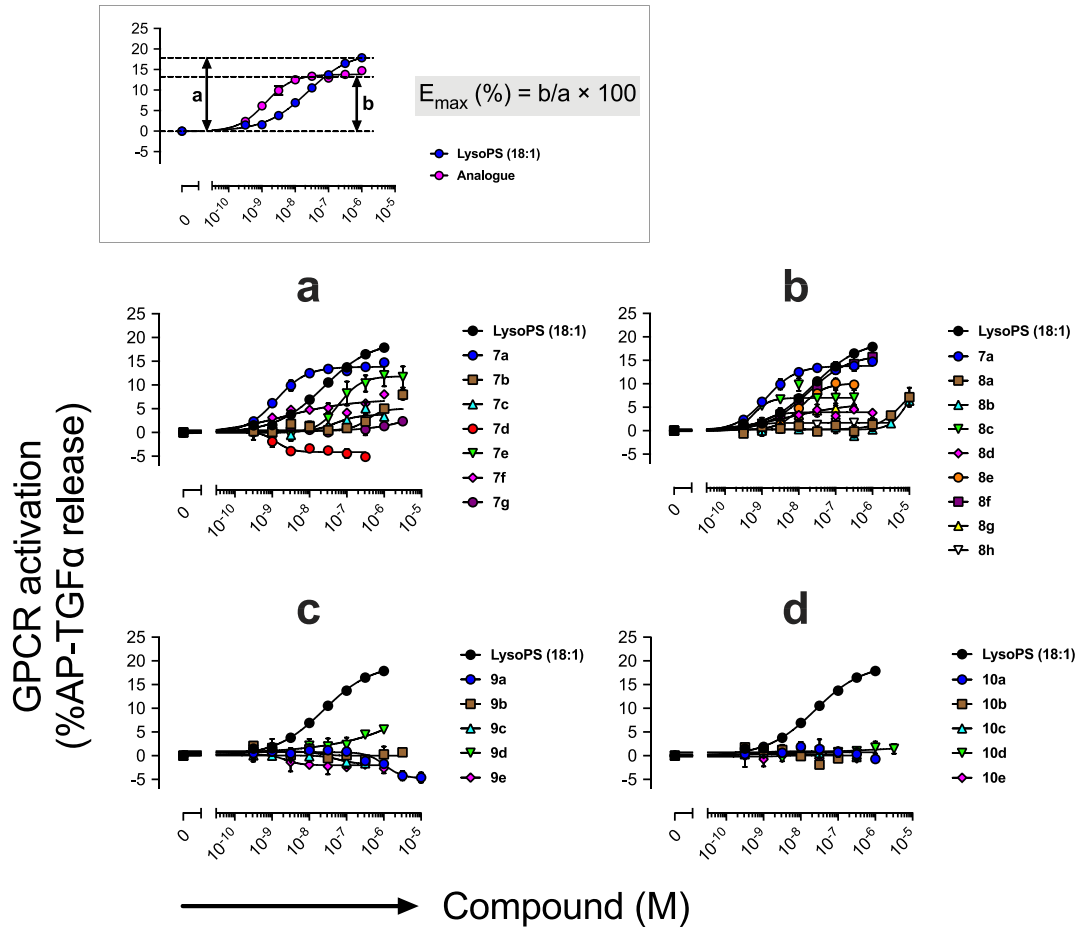

Figure 3. Agonistic activities of LysoPS analogues for GPR174. HEK293FT cells were transfected with mouse GPR174-coding plasmid or an empty plasmid and an AP-TGFα-coding plasmid and treated with LysoPS analogues. Receptor-specific GPCR activation (% AP-TGFα release) was determined by subtracting the background responses in the mock-transfected cells from the responses in the GPR174-transfected cells. Data are mean and SEM for several independent experiments indicated in Tables 1−4. The definition of E_{max} is also shown in the upper frame.

Methylation at the β-position of the serine carbonyl group in **7d** or **10a** decreased the GPR174 antagonistic activity: **10b** IC_{50} = 100 nM; **10c** IC_{50} = 120 nM; **10d** inactive; and **10e** inactive (Table 4 and Figures 4d and S3d). The agonistic activity was retained when the serine moiety of LysoPS was converted to *allo*-threonine.[18] However, introduction of a methyl group at the β-position of the serine carbonyl group in antagonist **7d** with the same stereochemistry as in *allo*-threonine (**10b**) greatly reduced the antagonistic activity compared with **7d** (**7d** IC_{50} = 5.6 nM and **10b** IC_{50} = 100 nM) (Table 4 and Figures 4d and S3d). Thus, the interaction of the hydrophilic moiety with the receptor appears to be different between agonists and antagonists.

Overall, our results suggest that all the fatty acid, glycerol, and phosphoserine moieties in GPR174 antagonists are recognized by the receptor, and therefore, agonists and antagonists may share the same binding pocket in GPR174.

Amide Functionality Is Placed Near TM7 in GPR174−7d Binding Models. In order to investigate the origin of the antagonistic efficacy of α-amide derivatives at the atomic level, we created binding models of **7d** and GPR174. As the structure of GPR174 has not been reported, we built a homology model of GPR174 based on the crystal structure of the closely related GPCR, LPA receptor 6 (LPA_6) (PDBID: 5XSZ).[21] As the LPA_6 crystal structure lacks a bound ligand and its binding pocket is supposed to take a wide-open conformation before activation by an agonist, the structure should be suitable for antagonist binding. Since our results (Table 3) suggest that agonists and antagonists share the same binding pocket, we docked the antagonist **7d** to GPR174 with reference to the binding position of the agonist in a GPR34 model that we reported previously.[20] We obtained two binding models (models 1 and 2) of GPR174 and **7d** after docking and optimization of the whole complex by molecular dynamics simulation (Figure 5, model 1: blue protein and yellow ligand; model 2: red protein and yellow ligand). The two receptor protein structures are comparable, and the fatty acid moiety of **7d** was placed around transmembrane helices (TMs) 4, 5, and 6 at the intracellular part of the binding pocket in both models. This is consistent with the experimental result that the fatty acid moiety in GPR174 is recognized by the receptor. On the other hand, the hydrophilic part of **7d** showed a larger difference between the binding models: in model 1, the acetamide part in **7d** points toward TM2, being placed at a more extracellular location than in model 2. In model 2, the acetamide moiety faces TM7 (Figure 5, right, and see Figure 6).

GPR174 is related to the purinergic receptor family. In the purinergic receptor P2Y12, for which both agonist- and antagonist-bound crystal structures are available,[22,23] it is considered that the inward movement of the extracellular parts of TMs 6 and 7 to the center of the binding pocket occurs after binding of an agonist. Similarly, in GPR174, we suspect that when the pocket around TM7 in model 2 (TM7 pocket, Figure 6a) is occupied by the α-amide moiety of LysoPS derivatives, the

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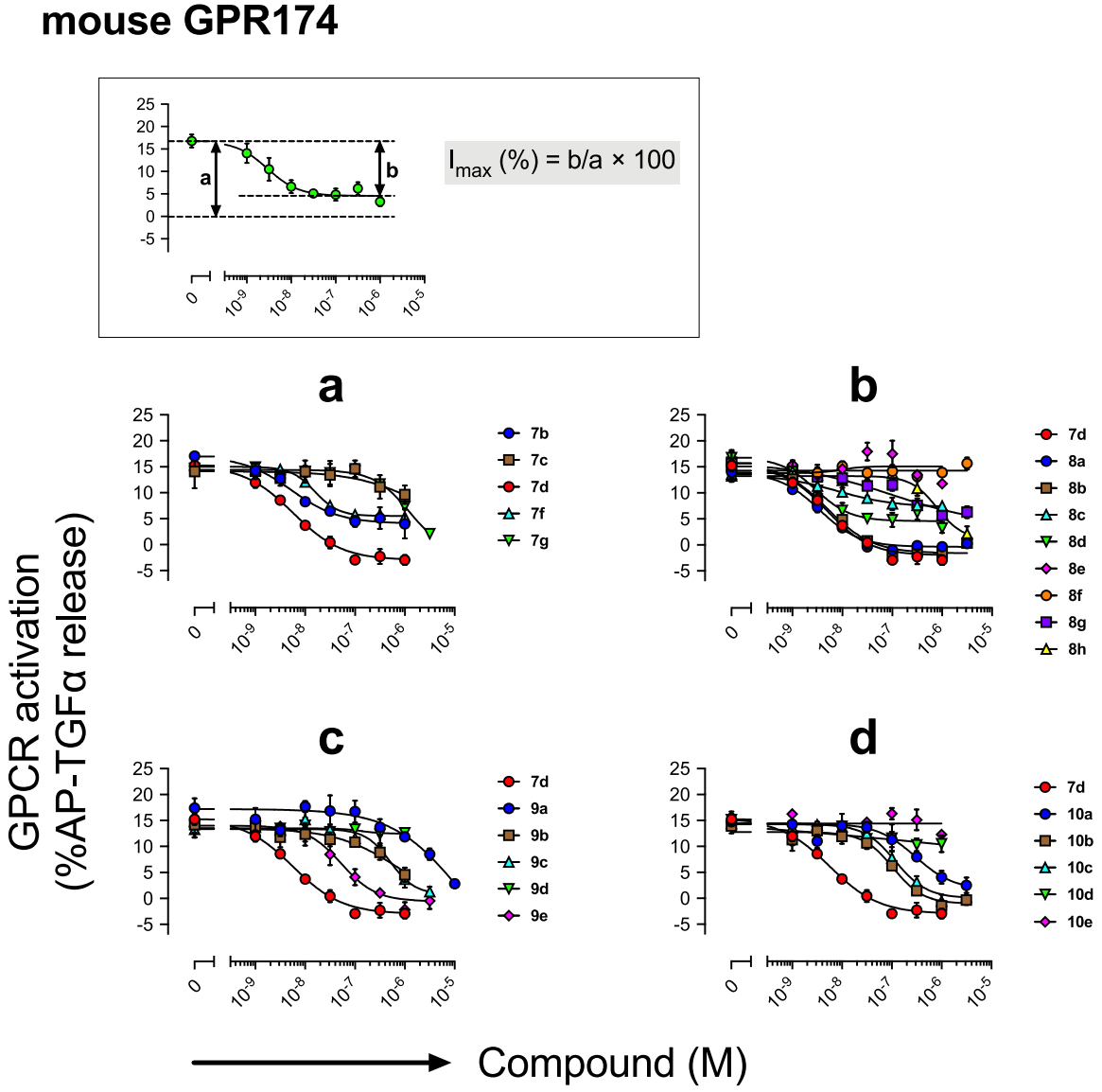

Figure 4. Antagonistic activities of LysoPS analogues for GPR174. The concentration−response curves of each LysoPS analogue were shown. HEK293FT cells transiently expressing both AP-TGFα and mouse GPR174 were treated with varying concentrations of test compounds in the presence of a GPR174 agonist, **LysoPalloT-NH-amide-C3-ph-m-O-C11** (100 nM). Cells expressing only AP-TGFα (GPR174 (−)) were used as a negative control. Receptor-specific AP-TGFα release was calculated by subtracting the responses in negative control cells from those in GPR174-expressing cells. Data are means and SEM for three independent experiments. Definition of I_{max} is also shown in the upper frame.

antagonistic activity may arise due to inhibition of the inward movement of TM7 (schematic view in Figure 6b). However, as the pocket around TM7 is not large (Figure 6a), extended amides such as those in **8c** (pentamide), **8e** (benzamide), and **8f** (cyclohexanecarboxyamide) are unlikely to be accommodated effectively in the TM7 pocket. Indeed, **8c**, **8e**, and **8f** showed agonistic activity rather than antagonistic activity (Table 2). From this perspective, model 2 seems more consistent with the experimental SAR than model 1, although further studies are necessary to better define the mechanism of agonist−antagonist switching.

CONCLUSIONS

In this study, we explored the structural requirements at the α-position of the serine moiety of LysoPS analogues for activity toward the receptor GPR174. We obtained highly potent and GPR174-selective antagonists (**7d**, **8a**, and **8b**) by attaching a short-chain alkylamide at the serine α-position. Furthermore, the agonist−antagonist characteristic of the analogues changed depending on the length and shape of the α-amide, affording partial agonists with varying values of E_{max} (E_{max}: **8d** < **8c** < **8e** < **8f**).

Every structural module of the obtained antagonists, that is, fatty acid, glycerol, and phosphoserine, appears to be recognized by the receptor, and our results suggest that agonists and antagonists occupy essentially the same binding position in the receptor. Modeling studies indicate that the efficacy of the LysoPS analogues depends on the ability of the amide group to block the inward movement of the extracellular part of the receptor, especially TM7. To our knowledge, this is the first report of potent GPR174 antagonists. We believe that these antagonists will be useful as tools to elucidate the immunological functions of GPR174, and they should also provide a good starting point for the development of candidate therapeutics for

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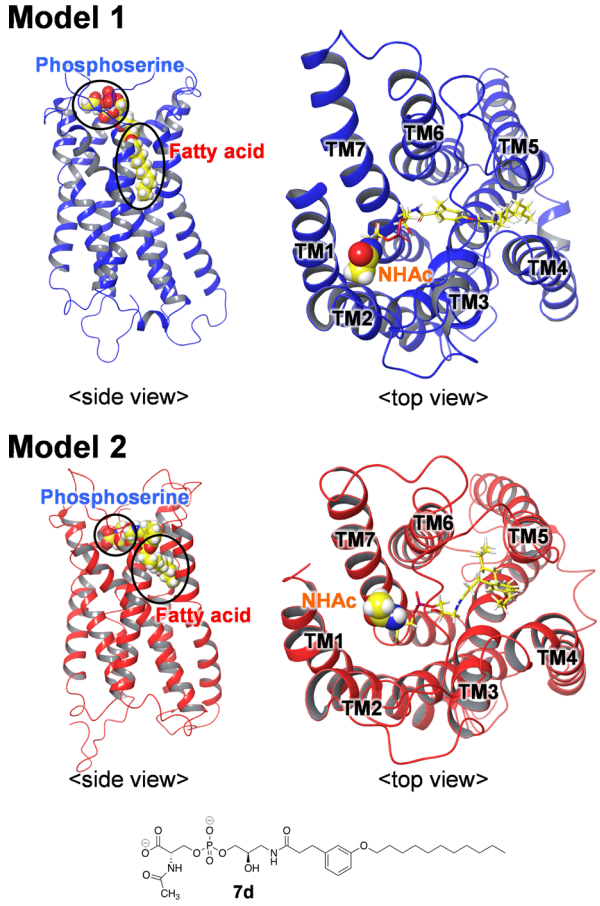


Figure 5. Two docking models of the antagonist (**7d**)−GPR174 complex. Model 1 (GPR174: blue ribbon) and model 2 (GPR174: red ribbon) were created on the basis on the crystal structure of LPA_6 (PDBID: 5XSZ). Views from the side (left) and from the top (right) are shown. The bound antagonist **7d** is shown as spheres or sticks (carbon: yellow, hydrogen: white, oxygen: red, nitrogen: blue, and phosphorus: purple). In the top view (right), only the acetamide part of the ligand **7d** is emphasized as spheres to indicate its different position in models 1 and 2.

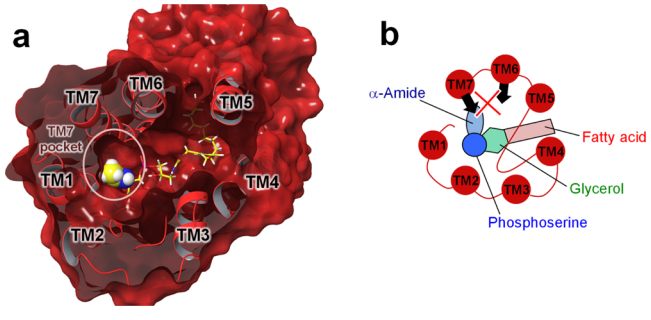


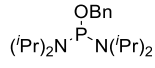
Figure 6. Putative inhibition of TM7 movement by the ligand α-amide moiety placed in the receptor TM7 pocket. (a) Top view of GPR174−**7d** complex model 2. The receptor surface is shown in addition to the ribbon representation of the receptor model (colored red). The antagonist **7d** is shown as sticks and its acetamide part is emphasized as spheres (carbon: yellow, hydrogen: white, oxygen: red, nitrogen: blue, and phosphorus: purple). (b) Schematic view of the putative inhibition of the inward movement of TM7 by antagonists. Top view of the receptor 7 TMs and the bound ligand is shown to indicate the proposed mechanism of agonist−antagonist switching.

autoimmune diseases. Further studies will be needed to establish in detail the mechanism that underlies the switch from agonist to antagonist activity in these analogues.

EXPERIMENTAL SECTION

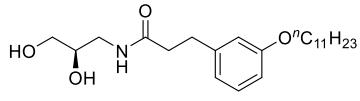
Melting points were determined with a Yanaco micro melting point apparatus without correction. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a Bruker AVANCE 400. The chemical shifts were calibrated with tetramethylsilane as an internal standard or with the solvent peak and are shown in ppm (δ) values; coupling constants are shown in hertz (Hz). The following abbreviations are used: s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, dd = double doublet, m = multiplet, and br s = broad singlet. An electron spray ionization time-of-flight (ESI-TOF) mass spectrometer (Bruker micrOTOF-05) was used to obtain high-resolution mass spectra (HRMS). All commercially available compounds and solvents were used as received. Combustion analyses were carried out in the Microanalytical Laboratory of the Graduate School of Pharmaceutical Sciences, the University of Tokyo. The purity of final compounds was examined by reverse-phase HPLC analysis: the purities of the final compounds, **7a**−**7g**, **8a**−**8d**, **8f**, **9b**−**9d**, and **10a**−**10e**, were ≥95%. The compounds with the purity below 95% were **8e** (88%), **8g** (94%), **8h** (86%), **9a** (94%), and **9e** (90%). The HPLC charts of the lead compounds (**7a**, **7d**, **8a**, **8c**, and **8f**) are provided in the Supporting Information (Figure S4). HPLC conditions: Imtakt Unison UK-C18 (Imtakt Corporation, Kyoto, Japan), 3 μm, 150 × 4.6 mm, acetonitrile/H_2O containing 0.1% HCO_2H as an eluent, detection: 210 nm UV, and flow rate 1.0 mL/min.

Synthesis of 7d. *Compound* ***3****.*



Bis(diisopropylamino)chlorophosphine (995.8 mg, 3.732 mmol) was dissolved in anhydrous Et_2O (10 mL). A solution of benzyl alcohol (405.9 mg, 3.753 mmol) and Et_3N (525 μL) was added dropwise at 0 °C, and the whole was stirred at 0 °C under an Ar atmosphere for 4 h. The solution was filtered through Celite, and the filtrate was evaporated to yield **3** as a colorless oil (1149.4 mg, 3.3958 mmol), which was used without further purification.

Compound ***11****.*



(*R*)-3-Amino-1,2-propanediol (1255.4 mg, 13.779 mmol), fatty acid surrogate **1** (4006.9 mg, 12.503 mmol), and 1-hydroxybenzotriazole monohydrate (2024.6 mg, 13.220 mmol) were dissolved in anhydrous DMF (40 mL). EDCI·HCl (2878.2 mg, 15.014 mmol) was added at 0 °C, and the whole was stirred at room temperature under an Ar atmosphere for 5.5 h. A saturated aqueous solution of $NaHCO_3$ (70 mL) was added. The mixture was filtered to obtain the precipitate **11**, a colorless powder (4734.3 mg, 12.029 mmol, 96%).

^{1}H NMR (400 MHz, $CDCl_3$): 7.208−2.167 (1H, m), 6.770−6.733 (3H, m), 6.005 (1H, t, J = 5.7 Hz), 3.924 (2H, t, J = 6.6 Hz), 3.680 (1H, quin, J = 5.1 Hz), 3.493−3.275 (4H, m), 2.927 (2H, t, J = 7.5 Hz), 2.766 (2H, br s), 2.515 (2H, t, J = 7.5 Hz), 1.761 (2H, quin, J = 7.0 Hz), 1.473−1.267 (16H, m), 0.881 (3H, t, J = 6.9 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): 174.15, 159.33, 141.96, 129.55, 120.46, 114.76, 112.21, 70.95, 67.96, 63.46, 42.16, 38.14, 31.89, 31.69, 29.60, 29.57, 29.41, 29.32, 29.29, 26.04, 22.67, 14.10. HRMS (ESI-TOF, $[M + Na]^+$): calcd for $C_{23}H_{39}NNaO_4^+$, 416.2771; found, 416.2742.

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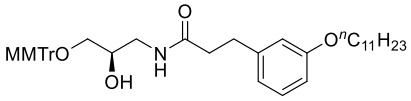
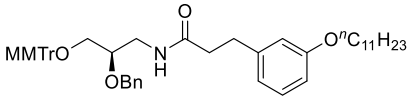
Compound ***12****.*



Compound **11** (4725.9 mg, 12.008 mmol) and DIPEA (6.2 mL, 36 mmol) were dissolved in anhydrous THF (40 mL). MMTrCl (4077.0 mg, 13.203 mmol) was added to the solution, and the whole was stirred at room temperature under an Ar atmosphere for 4.5 h. A saturated aqueous solution of $NaHCO_3$ (50 mL) was added. The water layer was separated and extracted twice with AcOEt (50 mL × 2). The combined organic layer was washed with brine, dried over Na_2SO_4 and filtered, and the solvent was evaporated. The residue was column-chromatographed on an open column with silica gel (*n*-hexane/AcOEt = 2:1, 1:1 to 2:3) to afford **12** (6840.0 mg, 10.272 mmol, 86%) as a yellow sticky oil.

^{1}H NMR (400 MHz, $CDCl_3$): 7.415−7.394 (4H, m), 7.309−7.258 (7H, m), 7.244−7.202 (2H, m), 7.186−7.146 (1H, m), 6.828 (2H, d, *J* = 8.9 Hz), 6.738−6.714 (3H, m), 5.579 (1H, t, *J* = 5.6 Hz), 3.913 (2H, t, J = 6.6 Hz), 3.841−3.828 (1H, m), 3.770 (3H, s), 3.550−3.490 (1H, m), 3.196−3.098 (3H, m), 3.027 (1H, d, *J* = 4.7 Hz), 2.850 (2H, t, *J* = 7.8 Hz), 2.371 (2H, t, *J* = 7.7 Hz), 1.755 (2H, quin, *J* = 7.1 Hz), 1.469−1.264 (16H, m), 0.880 (3H, t, *J* = 6.9 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): 173.31, 159.30, 158.64, 144.11, 144.06, 142.22, 135.21, 130.30, 129.45, 128.28, 127.91, 127.03, 120.39, 114.63, 113.18, 112.12, 86.48, 70.25, 67.88, 64.62, 55.19, 42.82, 38.20, 31.89, 31.73, 30.92, 29.60, 29.57, 29.40, 29.32, 29.30, 26.05, 22.67, 14.11. HRMS (ESI-TOF, $[M + Na]^+$): calcd for $C_{43}H_{55}NNaO_5^+$, 688.3972; found, 688.3989.

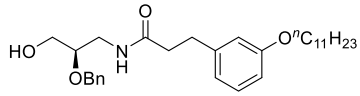
Compound ***13****.*



Compound **12** (6784.5 mg, 10.188 mmol) was dissolved in anhydrous THF (40 mL). NaH (60% dispersion in oil, 617.7 mg, 15.44 mmol) was added to the solution at 0 °C, and the mixture was stirred at 0 °C under an Ar atmosphere for 20 min. Benzyl bromide (1.6 mL) was added to the mixture at 0 °C, and the mixture was stirred at room temperature under an Ar atmosphere for 4.3 h. A saturated aqueous solution of $NaHCO_3$ (40 mL) was added. The water layer was separated and extracted twice with AcOEt (40 mL × 2). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered, and the solvent was evaporated. The residue was column-chromatographed on an open column with silica gel twice (first, *n*-hexane/AcOEt = 4:1 to 2:1, second, *n*-hexane/AcOEt = 4:1, 2:1 to 1:1) to afford **13** (6987.7 mg, 9.2425 mmol, 91%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$): 7.447−7.427 (4H, m), 7.362−7.280 (11H, m), 7.234−7.198 (2H, m), 7.167−7.127 (1H, m), 6.816 (2H, d, *J* = 8.9 Hz), 6.723−6.701 (3H, m), 5.616−5.601 (1H, m), 4.603 (1H, d, *J* = 11.7 Hz), 4.436 (1H, d, *J* = 11.7 Hz), 3.899 (2H, t, *J* = 6.6 Hz), 3.770 (3H, s), 3.634−3.573 (2H, m), 3.302−3.157 (3H, m), 2.827 (2H, t, *J* = 7.9 Hz), 2.323−2.283 (2H, m), 1.750 (2H, quin, *J* = 7.0 Hz), 1.466−1.258 (16H, m), 0.879 (3H, t, *J* = 6.9 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): 171.95, 156.26, 158.57, 144.18, 142.41, 138.19, 135.31, 130.30, 129.37, 128.48, 128.34, 127.90, 127.86, 127.82, 126.94, 120.37, 114.59, 113.13, 112.03, 86.54, 76.46, 71.86, 67.83, 63.70, 55.17, 40.60, 38.28, 31.88, 31.69, 30.91, 29.59, 29.56, 29.40, 29.31, 26.05, 22.66, 14.10. HRMS (ESI-TOF, $[M + Na]^+$): calcd for $C_{50}H_{61}NNaO_5^+$, 778.4442; found, 778.4427.

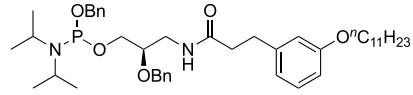
Compound ***2****.*



Compound **13** (299.6 mg, 0.3963 mmol) was dissolved in CH_2Cl_2 (1 mL) and MeOH (3 mL). *p*-TsOH·H_2O (7.9 mg, 0.042 mmol) was added, and the mixture was stirred at room temperature for 30 min. Et_3N (10 μL) was added to the mixture and the solution was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated. The residue was column-chromatographed on an open column with silica gel (*n*-hexane/AcOEt = 1:1 to 1:6) to afford **2** as a colorless solid (160.2 mg, 0.3312 mmol, 84%).

^{1}H NMR (400 MHz, $CDCl_3$): 7.368−7.257 (5H, m), 7.194−7.153 (1H, m), 6.761−6.724 (3H, m), 5.740 (1H, t, *J* = 5.8 Hz), 4.558 (1H, d, *J* = 11.8 Hz), 4.498 (1H, d, *J* = 11.8 Hz), 3.914 (2H, t, *J* = 6.6 Hz), 3.636−3.574 (1H, m), 3.553−3.505 (2H, m), 3.460−3.395 (1H, m), 3.337−3.278 (1H, m), 3.164−3.131 (1H, m), 2.909 (2H, t, *J* = 7.6 Hz), 2.467 (2H, t, *J* = 7.6 Hz), 1.757 (2H, quin, *J* = 7.0 Hz), 1.471−1.265 (16H, m), 0.880 (3H, t, *J* = 6.7 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): 173.49, 159.32, 142.08, 137.96, 129.49, 128.56, 128.00, 127.81, 120.43, 114.64, 112.22, 77.54, 71.70, 67.89, 60.75, 39.37, 38.19, 31.89, 31.65, 29.59, 29.57, 29.40, 29.32, 29.30, 26.05, 22.67, 14.10. HRMS (ESI-TOF, $[M + Na]^+$): calcd for $C_{30}H_{45}NNaO_4^+$, 506.3241; found, 506.3238. Anal. Calcd for $C_{30}H_{45}NO_4$: C, 74.50; H, 9.38; N, 2.90. Found: C, 74.30; H, 9.25; N, 2.81. mp 43.0−44.8 °C (colorless solids, recrystallized from *n*-hexane/CH_2Cl_2).

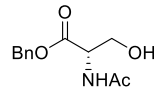
Compound ***4****.*



Compound **2** (485.9 mg, 1.005 mmol) was dissolved in CH_2Cl_2 and toluene. To remove traces of water, the solution was evaporated. Compound **3** (crude, 585.4 mg, 1.730 mmol) was added, and the mixture was dissolved in anhydrous CH_2Cl_2 (4.0 mL). A solution of 1*H*-tetrazole (76.1 mg, 1.09 mmol) in anhydrous THF (4.0 mL) was added at 0 °C. The mixture was stirred at room temperature under an Ar atmosphere for 7.4 h. A saturated aqueous solution of $NaHCO_3$ (10 mL) was added. The whole was extracted three times with CH_2Cl_2 (10 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered, and the solvent was evaporated. The residue was column-chromatographed on an open column with silica gel (*n*-hexane/AcOEt/Et_3N = 40:1:1 to 40:8:1) to afford **4** (crude) as a colorless oil (586.1 mg, 0.8129 mmol, 81%).

HRMS (ESI-TOF, $[M + H]^+$): calcd for $C_{43}H_{66}N_2O_5P^+$, 721.4704; found, 721.4696.

Compound ***5****.*



L-Serine benzyl ester hydrochloride (693.8 mg, 2.995 mmol) was dissolved in anhydrous CH_2Cl_2 (10 mL). Et_3N (1040 μL) and acetyl chloride (235 μL, 3.31 mmol) were added, and the mixture was stirred at room temperature under an Ar atmosphere for 1.5 h. MeOH (ca. 2 mL) was added, and the solvent was evaporated with silica gel. The residue was column-chromatographed on an open column with silica gel (*n*-hexane/acetone = 2:1 to 1:1) to afford **5** as a colorless solid (637.9 mg, 2.689 mmol, 90%).

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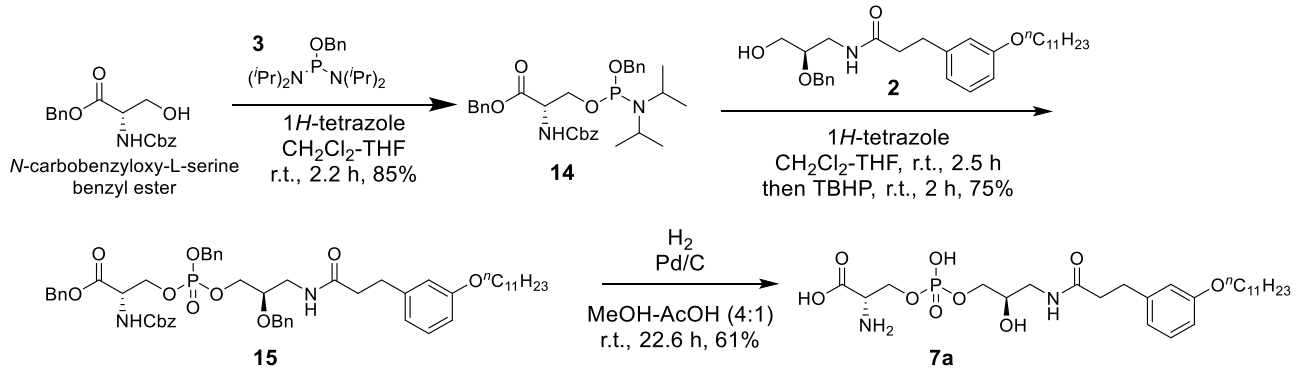
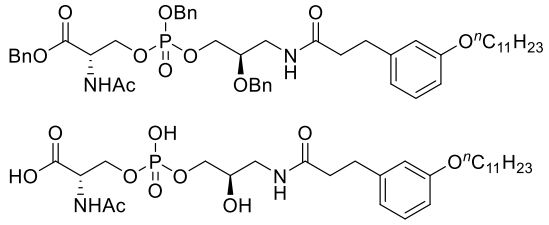


Figure 7. Synthesis of **7a**.

^{1}H NMR (400 MHz, $CDCl_3$): 7.401−7.314 (5H, m), 6.524 (1H, d, J = 6.4 Hz), 5.217 (2H, s), 4.727−4.691 (1H, m), 4.007−3.980 (1H, m), 3.934−3.906 (1H, m), 2.670 (1H, br s), 2.054 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$): 170.64, 170.36, 135.06, 128.66, 128.56, 128.16, 67.54, 63.47, 54.87, 23.10. HRMS (ESI-TOF, [M + Na]$^+$): calcd for $C_{12}H_{15}NNaO_4^+$, 260.0893; found, 260.0909. mp 82.0−82.5 °C (colorless needles, recrystallized from *n*-hexane/CH_2Cl_2).

*Compounds **6** and **7d**.*

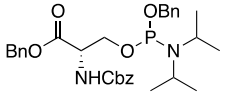


Compounds **5** (47.2 mg, 0.199 mmol) and **4** (233.4 mg, 0.3237 mmol) were dissolved in CH_2Cl_2 and toluene. To remove traces of water, the solution was evaporated. The mixture was dissolved in anhydrous CH_2Cl_2 (1.0 mL). A solution of 1*H*-tetrazole (27.8 mg, 0.397 mmol) in anhydrous THF (1.0 mL) was added at 0 °C. The mixture was stirred at room temperature under an Ar atmosphere for 3 h. TBHP in decane (5.0−6.0 M) (73 μL) was added, and the mixture was stirred at room temperature for 1.5 h. H_2O (10 mL) was added, and the whole was extracted three times with CH_2Cl_2 (10 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated to afford a colorless oil, which was column-chromatographed on a flash column with silica gel ($CHCl_3$/AcOEt = 1:1 to *n*-hexane/acetone = 2:3) to afford crude **6** as a colorless oil (109.7 mg).

Crude **6** (106.4 mg) and Pd/C (18.2 mg) were dissolved in THF (5 mL). The mixture was stirred at room temperature under a H_2 atmosphere for 5 h, then filtered through Celite, and the solvent was evaporated. The residue was purified by reversed-phase medium-pressure liquid chromatography (CH_3CN/H_2O = 3:7 to 7:3, containing 0.1% HCOOH). The solid was vacuum-dried at 40 °C to afford **7d** (46.6 mg, 0.0773 mmol, 39%, two steps).

^{1}H NMR (400 MHz, DMSO-d_6): 8.385 (1H, d, J = 7.8 Hz), 7.886 (1H, t, J = 5.6 Hz), 7.149 (1H, dd, J = 8.1 Hz, 8.1 Hz), 6.754−6.699 (3H, m), 4.480−4.439 (1H, m), 4.110−3.993 (2H, m), 3.910 (2H, t, J = 6.5 Hz), 3.746−3.675 (2H, m), 3.657−3.595 (1H, m), 3.199−3.123 (1H, m), 3.054−2.990 (1H, m), 2.757 (2H, t, J = 7.9 Hz), 2.397−2.358 (2H, m), 1.869 (3H, s), 1.683 (2H, quin, J = 6.9 Hz), 1.392−1.356 (2H, m), 1.247 (14H, br s), 0.854 (3H, t, J = 6.9 Hz). ^{31}P NMR (161 MHz, DMSO-d_6): −0.92. HRMS (ESI-TOF, [M − H]$^-$): calcd for $C_{28}H_{46}N_2O_{10}P^-$, 601.2896; found, 601.2914. HPLC: 97% (CH_3CN/H_2O = 1:1 (0.1% HCO_2H)).

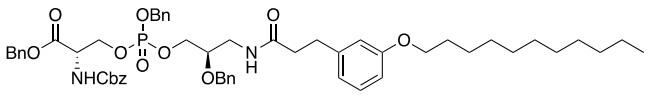
Synthesis of 7a (Figure 7). *Compound **14**.*



N-Carbobenzyloxy-L-serine benzyl ester (495.1 mg, 1.503 mmol) was dissolved in CH_2Cl_2 and toluene. To remove traces of water, the solution was evaporated. **3** (crude, 931.7 mg, 2.753 mmol) was added, and the mixture was dissolved in anhydrous CH_2Cl_2 (6.0 mL). A solution of 1*H*-tetrazole (111.0 mg, 1.584 mmol) in anhydrous THF (6.0 mL) was added at 0 °C. The mixture was stirred at room temperature under an Ar atmosphere for 2.2 h. A saturated aqueous solution of $NaHCO_3$ (15 mL) was added. The aqueous layer was separated and extracted twice with CH_2Cl_2 (15 mL × 2). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated. The residue was column-chromatographed on an open column with silica gel (*n*-hexane/AcOEt/Et_3N = 40:2:1, 40:3:1, 40:4:1, to 40:5:1) to afford **14** as a colorless oil (724.8 mg, 1.279 mmol, 85%) (Figure 7).

^{1}H NMR (400 MHz, $CDCl_3$): 7.354−7.223 (15H, m), 5.898 (J = 8.6 Hz) and 5.669 (J = 8.5 Hz) (1H, 2d), 5.168−5.085 (4H, m), 4.721−4.511 (3H, m), 4.173−4.123 (1H, m), 3.942−3.848 (1H, m), 3.610−3.511 (2H, m), 1.150−1.099 (12H, m). ^{13}C NMR (100 MHz, $CDCl_3$): 169.91, 155.99, 155.85, 139.09, 138.89, 136.30, 136.23, 135.33, 135.21, 128.45, 128.41, 128.39, 128.23, 128.19, 128.12, 128.03, 127.98, 127.94, 127.34, 127.24, 127.03, 126.84, 67.20, 67.11, 66.88, 66.82, 65.44, 65.37, 65.26, 65.19, 64.16, 64.01, 63.66, 63.51, 55.37, 55.31, 55.24, 55.17, 43.09, 42.96, 30.83, 24.54, 24.48, 24.41, 24.34. HRMS (ESI-TOF, [M + Na]$^+$): calcd for $C_{31}H_{40}N_2O_6P^+$, 567.2618; found, 567.2644.

*Compound **15**.*



Compounds **14** (180.4 mg, 0.3184 mmol) and **2** (99.1 mg, 0.205 mmol) were dissolved in CH_2Cl_2 and toluene. To remove traces of water, the solution was evaporated. The mixture was dissolved in anhydrous CH_2Cl_2 (2.0 mL). A solution of 1*H*-tetrazole (29.3 mg, 0.418 mmol) in anhydrous THF (2.0 mL) was added. The mixture was stirred at room temperature under an Ar atmosphere for 2.5 h. TBHP in decane (5.0−6.0 M) (75 μL) was added, and the mixture was stirred at room temperature for 2 h. H_2O (10 mL) was added, and the whole was extracted three times with CH_2Cl_2 (10 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated to afford a colorless oil. The residue was column-chromatographed on a flash column with silica gel

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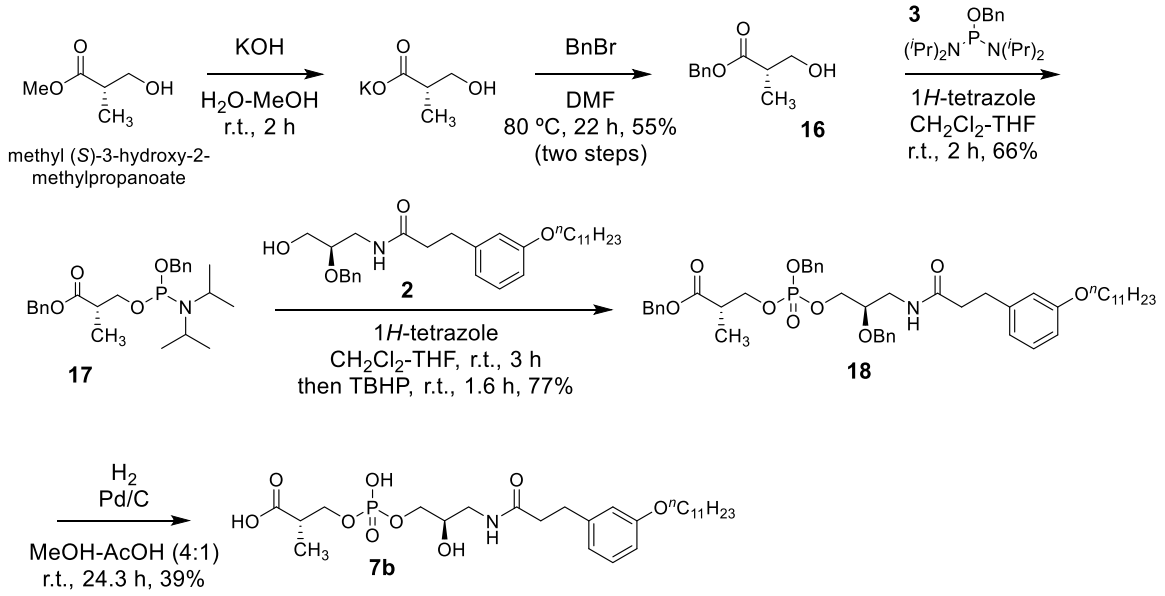
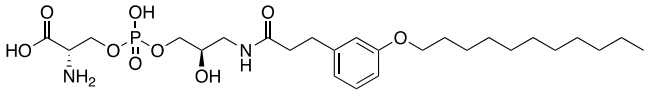


Figure 8. Synthesis of **7b**.

($CHCl_3$/AcOEt = 4:1) to afford **15** as a colorless oil (148.4 mg, 0.1538 mmol, 75%).

^{1}H NMR (400 MHz, $CDCl_3$): (mixtures of diastereomers) 7.309−7.270 (20H, m), 7.165−7.120 (1H, m), 6.759−6.696 (3H, m), 6.073−6.022 (1H, m), 5.857−5.813 (1H, m), 5.199−5.054 (4H, m), 5.026−4.945 (2H, m), 4.612−4.544 (2H, m), 4.438−4.397 (2H, m), 4.340−4.212 (1H, m), 4.038−3.799 (4H, m), 3.645−3.528 (1H, m), 3.372−3.256 (2H, m), 2.838 (2H, t, J = 6.8 Hz), 2.437−2.270 (2H, m), 1.743 (2H, quin, J = 6.9 Hz), 1.462−1.237 (16H, m), 0.878 (3H, t, J = 6.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): (mixtures of diastereomers) 172.31, 168.77, 159.25, 155.90, 142.31, 137.52, 136.00, 134.91, 129.37, 128.77, 128.64, 128.60, 128.51, 128.22, 128.09, 128.01, 120.43, 114.67, 112.03, 75.34, 71.90, 71.84, 69.80, 67.84, 67.71, 67.51, 67.17, 54.52, 39.13, 39.04, 38.01, 31.88, 31.53, 30.90, 29.59, 29.56, 29.40, 29.30, 26.04, 22.65, 14.09. HRMS (ESI-TOF, [M + NH_4]$^+$): calcd for $C_{55}H_{73}N_3O_{11}P^+$, 982.4977; found, 982.4998.

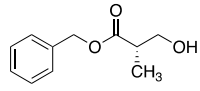
*Compound **7a**.*



Compound **15** (119.9 mg, 0.1242 mmol) and Pd/C (10.5 mg) were dissolved in MeOH (4.0 mL) and AcOH (1.0 mL). The mixture was stirred at room temperature under a H_2 atmosphere for 22.6 h. The mixture was filtered on Celite, and the solvent was evaporated. The residue was purified by reversed-phase medium-pressure liquid chromatography (CH_3CN/H_2O = 2:8 to 7:3, containing 0.1% HCOOH). The solid was vacuum-dried at 40 °C to afford **7a** as a colorless powder (42.3 mg, 0.0755 mmol, 61%).

^{1}H NMR (400 MHz, DMSO-d_6): 8.725 (2H, br s), 7.938 (1H, t, J = 5.6 Hz), 7.147 (1H, dd, J = 8.0 Hz, 8.0 Hz), 6.752−6.697 (3H, m), 4.071−4.043 (3H, m), 3.911 (2H, t, J = 6.5 Hz), 3.599−3.549 (3H, m), 3.160−3.126 (1H, m), 3.042−2.994 (1H, m), 2.754 (2H, t, J = 7.8 Hz), 2.366 (2H, t, J = 7.9 Hz), 1.683 (2H, quin, J = 6.9 Hz), 1.428−1.247 (16H, m), 0.854 (3H, t, J = 6.8 Hz). HRMS (ESI-TOF, [M − H]$^-$): calcd for $C_{26}H_{44}N_2O_9P^-$, 559.2790; found, 559.2791. HPLC: 98% (CH_3CN/H_2O = 1:1 (0.1% HCO_2H)).

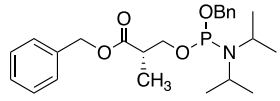
Synthesis of 7b (Figure 8). *Compound **16**.*



Methyl (*S*)-(+)-3-hydroxyisobutyrate (249.9 mg, 2.115 mmol) was dissolved in MeOH (2 mL). An aqueous solution of KOH (1.95 M, 2 mL) was added, and the whole was stirred at room temperature for 2 h. The solvent was evaporated. The residue was dissolved in anhydrous DMF (3 mL) and benzyl bromide (245 μL) was added to the mixture. The mixture was heated to 80 °C under an Ar atmosphere for 22 h. An aqueous solution of HCl (2 M, 5 mL) and H_2O (10 mL) were added, and the whole was extracted three times with AcOEt (10 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered, and the solvent was evaporated. The residue was column-chromatographed on a flash column with silica gel (*n*-hexane/AcOEt = 7:2) to afford **16** as a colorless oil (226.2 mg, 1.165 mmol, 55%) (Figure 8).

^{1}H NMR (400 MHz, $CDCl_3$): 7.397−7.307 (5H, m), 5.162 (2H, s), 3.780−3.695 (2H, m), 2.774−2.690 (1H, m), 1.205 (3H, d, J = 7.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): 175.44, 135.75, 128.59, 128.27, 128.02, 66.40, 64.52, 41.75, 13.39. HRMS (ESI-TOF, [M + Na]$^+$): calcd for $C_{11}H_{14}NaO_3^+$, 217.0835; found, 217.0847.

*Compound **17**.*



Compound **16** (180.3 mg, 0.9289 mmol) was dissolved in CH_2Cl_2 and toluene. To remove traces of water, the solution was evaporated. **3** (678.0 mg, 2.003 mmol) was added, and the mixture was dissolved in anhydrous CH_2Cl_2 (3.0 mL). A solution of 1*H*-tetrazole (67.9 mg, 0.969 mmol) in anhydrous THF (3.0 mL) was added at 0 °C. The mixture was stirred at room temperature under an Ar atmosphere for 2 h. A saturated aqueous solution of $NaHCO_3$ (10 mL) was added and the aqueous layer was extracted three times with CH_2Cl_2 (10 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated to afford

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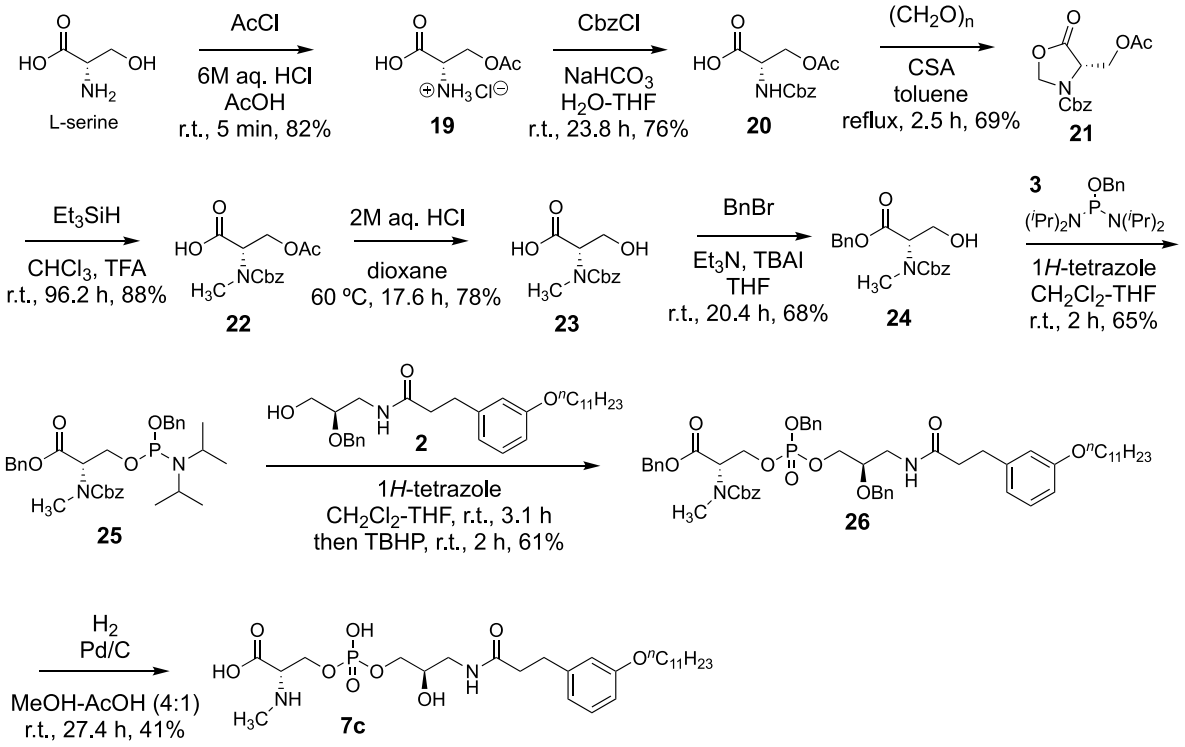
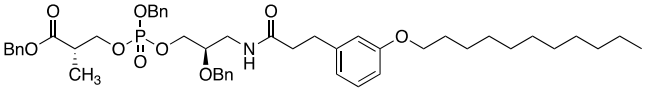


Figure 9. Synthesis of **7c**.

a colorless oil. The residue was column-chromatographed on an open column with silica gel (*n*-hexane/AcOEt/Et_3N = 40:1:1) to afford **17** as a colorless oil (265.8 mg, 0.6160 mmol, 66%).

^{1}H NMR (400 MHz, $CDCl_3$): 7.344−7.230 (10H, m), 5.156−5.076 (2H, m), 4.739−4.681 (1H, m), 4.657−4.604 (1H, m), 3.940−3.562 (4H, m), 2.863−2.776 (1H, m), 1.223−1.196 (3H, m), 1.173−1.153 (12H, m). ^{13}C NMR (100 MHz, $CDCl_3$): 174.42, 174.35, 139.48, 136.07, 128.47, 128.20, 128.05, 128.03, 127.99, 127.95, 127.18, 126.92, 66.17, 66.15, 65.46, 65.38, 65.28, 65.21, 65.11, 43.08, 42.95, 41.54, 41.47, 24.61, 24.58, 24.55, 24.51, 13.83, 13.79. HRMS (ESI-TOF, $[M + H]^+$): calcd for $C_{24}H_{35}NO_4P^+$, 432.2298; found, 432.2313.

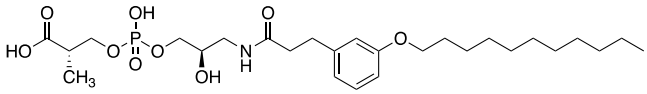
*Compound **18**.*



Compounds **17** (92.5 mg, 0.214 mmol) and **2** (51.6 mg, 0.137 mmol) were dissolved in CH_2Cl_2 and toluene. To remove traces of water, the solution was evaporated. The residue was dissolved in anhydrous CH_2Cl_2 (1.0 mL) and a solution of 1*H*-tetrazole (19.3 mg, 0.275 mmol) in anhydrous THF (1.0 mL) was added. The mixture was stirred at room temperature under an Ar atmosphere for 3 h. TBHP in decane (5.0−6.0 M) (50 μL) was added, and the mixture was stirred at room temperature for 1.6 h. H_2O (10 mL) was added, and the whole was extracted three times with CH_2Cl_2 (10 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated to afford a colorless oil. The residue was column-chromatographed on a flash column with silica gel ($CHCl_3$/AcOEt = 4:1) to afford **18** as a colorless oil (87.4 mg, 0.105 mmol, 77%).

^{1}H NMR (400 MHz, $CDCl_3$): (mixtures of diastereomers) 7.354−7.274 (15H, m), 7.171−7.124 (1H, m), 6.748−6.696 (3H, m), 6.022−5.955 (1H, m), 5.143−5.073 (2H, m), 5.032−4.986 (2H, m), 4.588 (1H, d, *J* = 11.7 Hz), 4.477 (1H, dd, *J* = 11.6 Hz, 3.5 Hz), 4.229−4.135 (1H, m), 4.099−3.961 (2H, m), 3.930−3.872 (3H, m), 3.619−3.553 (1H, m), 3.419−3.305 (2H, m), 2.892−2.777 (3H, m), 2.408−2.364 (2H, m), 1.778−1.725 (2H, m), 1.464−1.392 (2H, m), 1.346−1.263 (14H, m), 1.186−1.146 (3H, m), 0.879 (3H, t, *J* = 6.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): (mixtures of diastereomers) 173.17, 172.27, 172.25, 159.26, 142.36, 137.74, 135.62, 129.37, 128.66, 128.61, 128.60, 128.55, 128.48, 128.26, 128.05, 128.00, 127.96, 127.95, 120.42, 114.65, 112.05, 75.46, 75.40, 71.85, 69.58, 69.52, 68.73, 68.68, 67.84, 66.59, 66.57, 66.37, 40.43, 40.35, 38.96, 38.14, 31.88, 31.63, 29.59, 29.56, 29.40, 29.31, 26.05, 22.66, 14.10, 13.39, 13.36. HRMS (ESI-TOF, $[M + Na]^+$): calcd for $C_{48}H_{64}NNaO_9P^+$, 852.4211; found, 852.4239. Anal. Calcd for $C_{48}H_{64}NO_9P{\cdot}0.5H_2O$: C, 68.71; H, 7.81; N, 1.67. Found: C, 68.71; H, 7.79; N, 1.63.

*Compound **7b**.*



Compound **18** (70.9 mg, 0.0854 mmol) and Pd/C (8.0 mg) were dissolved in MeOH (4 mL) and AcOH (1 mL). The mixture was stirred at room temperature under a H_2 atmosphere for 24.3 h. The mixture was filtered on Celite, and the solvent was evaporated. The residue was purified by reversed-phase medium-pressure liquid chromatography (CH_3CN/H_2O = 1:1 to 7:3, containing 0.1% HCOOH). The residue was column-chromatographed on a flash column with silica gel ($CHCl_3$/MeOH/AcOH = 8:1:1 to 7:1:2) and vacuum-dried at 40 °C to afford **7b** (18.8 mg, 0.0336 mmol, 39%).

^{1}H NMR (400 MHz, DMSO-d_6): 7.988 (1H, br s), 7.142 (1H, dd, *J* = 8.1 Hz, 8.1 Hz), 6.753−6.698 (3H, m), 3.910 (2H, t, *J* = 6.5 Hz), 3.694 (2H, br s), 3.596−3.572 (2H, m), 3.516−3.507 (1H, m), 3.075−3.025 (2H, m), 2.752 (2H, t, *J* = 7.8 Hz), 2.360 (2H, t, *J* = 7.9 Hz), 1.682 (2H, quin, *J* = 6.9 Hz), 1.393−1.248 (16H, m), 0.978 (3H, d, *J* = 7.0 Hz), 0.854 (3H, t, *J* = 6.8 Hz). HRMS (ESI-TOF, $[M - H]^-$): calcd for $C_{27}H_{45}NO_9P^-$, 558.2837; found, 558.2866. HPLC: 98% (CH_3CN/H_2O = 3:2 (0.1% HCO_2H)).

https://doi.org/10.1021/acs.jmedchem.1c00347
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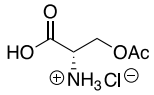
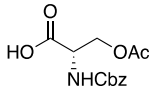
Synthesis of 7c (Figure 9). *Compound* ***19****.*



L-Serine (525.8 mg, 5.003 mmol) was dissolved in 6 M aqueous solution of HCl (1 mL) and AcOH (10 mL). The whole was stirred at room temperature for 5 min. Acetyl chloride (10 mL) was then added to the solution dropwise at 0 °C via 7 min, and the mixture was stirred at 0 °C for 5 min. Et_2O (25 mL) was added. A colorless solid, **19**, was obtained by filtration (750.4 mg, 4.087 mmol, 82%) (Figure 9).

^{1}H NMR (400 MHz, DMSO-d_6): 14.076 (1H, br s), 8.695 (3H, br s), 4.488 (1H, dd, J = 11.9 Hz, 3.2 Hz), 4.346 (1H, dd, J = 11.9 Hz, 4.6 Hz), 4.288 (1H, t, J = 3.6 Hz), 2.049 (3H, s). ^{13}C NMR (100 MHz, DMSO-d_6): 169.91, 168.33, 61.45, 51.24, 20.61. HRMS (ESI-TOF, [M + H]$^+$): calcd for $C_5H_{10}NO_4^+$, 148.0604; found, 148.0632.

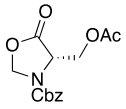
Compound ***20****.*



Compound **19** (740.2 mg, 4.032 mmol) was dissolved in THF (10 mL) and a saturated solution of $NaHCO_3$ (7.5 mL). Benzyl chloroformate (828.4 mg, 4.856 mmol) in 5 mL of THF was added to the solution, and the whole was stirred at room temperature for 23.8 h. H_2O (10 mL) and 2 M aqueous solution of HCl (3 mL) were added, and the whole was extracted three times with AcOEt (20 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated to afford a yellow oil. The residue was column-chromatographed on an open column with silica gel (*n*-hexane/acetone = 2:1 to 0:1) to afford **20** (865.4 mg, 3.077 mmol, 76%).

^{1}H NMR (400 MHz, $CDCl_3$): 7.389−7.302 (5H, m), 5.632 (1H, d, J = 8.2 Hz), 5.163−5.099 (2H, m), 4.681−4.642 (1H, m), 4.493 (1H, dd, J = 11.5 Hz, 3.8 Hz), 4.401 (1H, dd, J = 11.3 Hz, 3.5 Hz), 4.293 (4H, br s), 2.046 (3H, s).

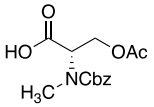
Compound ***21****.*



Compound **20** (834.7 mg, 2.968 mmol) was dissolved in anhydrous toluene (35 mL). Paraformaldehyde (1004.8 mg) and CSA (71.2 mg, 0.307 mmol) were added to the solution. The whole was heated to reflux and stirred under an Ar atmosphere for 2.5 h. The mixture was filtered on Celite. To the filtrate, Et_2O (15 mL) and a saturated solution of $NaHCO_3$ (20 mL) were added. The organic layer was separated and washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated to afford a yellow oil. The residue was column-chromatographed on an open column with silica gel (*n*-hexane/AcOEt = 3:1) to afford **21** as a colorless oil (600.9 mg, 2.049 mmol, 69%).

^{1}H NMR (400 MHz, $CDCl_3$): 7.415−7.337 (5H, m), 5.583 (1H, br s), 5.255 (1H, dd, J = 4.2 Hz, 0.9 Hz), 5.206 (2H, s), 4.648 (1H, br s), 4.445−4.362 (2H, m), 2.044 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$): 169.93, 152.36, 135.08, 128.70, 128.38, 78.61, 68.22, 62.34, 54.50, 20.56.[a] HRMS (ESI-TOF, [M + Na]$^+$): calcd for $C_{14}H_{15}NNaO_6^+$, 316.0792; found, 316.0794.

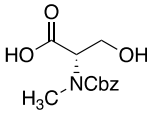
Compound ***22****.*



Compound **21** (571.4 mg, 1.948 mmol) was dissolved in $CHCl_3$ (10 mL). Et_3SiH (0.93 mL) and TFA (10 mL) were added to the solution, and the whole was stirred at room temperature for 96.2 h. Toluene (5 mL) was added and evaporated twice. The residue was column-chromatographed on an open column with silica gel (*n*-hexane/acetone = 2:1 to 0:1) to afford **22** as a colorless oil (507.4 mg, 1.718 mmol, 88%).

^{1}H NMR (400 MHz, $CDCl_3$): 7.365−7.291 (5H, m), 7.136 (2H, br s), 5.225−5.077 (2H, m), 4.988−4.956 and 4.892−4.858 (1H, 2m), 4.581−4.348 (2H, m), 2.972 (3H, s), 2.014 and 1.977 (3H, 2s) (two rotamers). ^{13}C NMR (100 MHz, $CDCl_3$): 172.52, 172.46, 170.72, 170.67, 156.94, 155.99, 136.10, 135.89, 128.48, 128.16, 128.12, 127.94, 127.77, 67.90, 67.85, 61.26, 58.46, 58.15, 32.78, 32.56, 20.64, 20.56 (two rotamers). HRMS (ESI-TOF, [M − H]$^-$): calcd for $C_{14}H_{16}NO_6^-$, 294.0983; found, 294.0987.

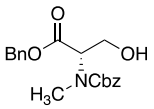
Compound ***23****.*



Compound **22** (504.2 mg, 1.707 mmol) was dissolved in dioxane (9.0 mL) and 2 M aqueous solution of HCl (9.0 mL). The mixture was stirred at 60 °C for 17.6 h. An aqueous solution of HCl (1 M, 20 mL) was added, and the whole was extracted three times with AcOEt (20 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated to afford a colorless oil. The residue was column-chromatographed on an open column with silica gel (*n*-hexane/acetone = 2:1 to 1:1) to afford **23** as a colorless oil (335.9 mg, 1.326 mmol, 78%).

^{1}H NMR (400 MHz, $CDCl_3$): 7.398−7.310 (5H, m), 6.069 (2H, br s), 5.150 (2H, s), 4.589 (1H, t, J = 6.0 Hz), 4.122−3.852 (2H, m), 3.002 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$): 173.87, 173.25, 157.42, 135.97, 128.52, 128.16, 127.93, 127.81, 67.96, 61.88, 61.00, 60.73, 33.69, 33.35 (two rotamers). HRMS (ESI-TOF, [M − H]$^-$): calcd for $C_{12}H_{14}NO_5^-$, 252.0877; found, 252.0887.

Compound ***24****.*



Compound **23** (310.1 mg, 1.224 mmol) was dissolved in anhydrous THF (12 mL). Triethylamine (510 μL), TBAI (136.2 mg, 0.3697 mmol), and BnBr (160 μL) were added at 0 °C, and the mixture was stirred at room temperature under an Ar atmosphere for 20.4 h. H_2O (20 mL) was added, and the whole was extracted three times with AcOEt (15 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated to afford a yellow oil. The residue was column-chromatographed on an open column with silica gel (*n*-hexane/AcOEt = 3:2) to afford **24** as a colorless oil (285.2 mg, 0.8306 mmol, 68%).

^{1}H NMR (400 MHz, $CDCl_3$): 7.341−7.271 (5H, m), 5.232−4.995 (4H, m), 4.609 and 4.509 (1H, 2t, J = 5.0 Hz and 6.5 Hz), 4.133−4.063 (1H, m), 3.979−3.963 and 3.822 (1H, m and br s), 2.971 and 2.964 (3H, 2s), 2.643 and 2.260 (1H, 2br s), (two rotamers). ^{13}C NMR (100 MHz, $CDCl_3$): 170.03, 169.75, 157.01, 155.93, 136.21, 136.00, 135.20, 135.01, 128.51, 128.40, 128.30, 128.10, 128.07, 127.98, 127.88, 127.69,

https://doi.org/10.1021/acs.jmedchem.1c00347
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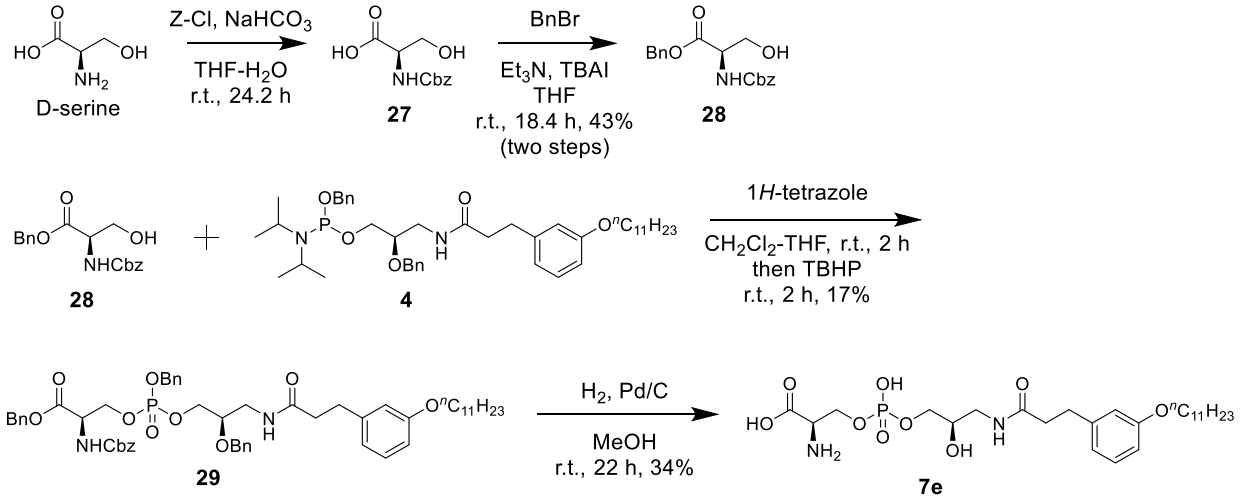
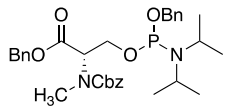


Figure 10. Synthesis of **7e**.

67.54, 66.99, 61.93, 61.45, 60.77, 33.46, 33.22 (two rotamers). HRMS (ESI-TOF, $[M + H]^+$): calcd for $C_{19}H_{22}NO_5^+$, 344.1492; found, 344.1503.

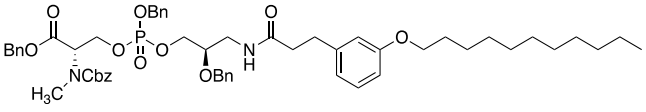
*Compound **25**.*



Compound **24** (124.0 mg, 0.3611 mmol) was dissolved in CH_2Cl_2 and toluene. To remove traces of water, the solution was evaporated. Compound **3** (crude, 260.0 mg, 0.7681 mmol) was added, and the mixture was dissolved in anhydrous CH_2Cl_2 (1.25 mL). A solution of 1*H*-tetrazole (26.9 mg, 0.384 mmol) in anhydrous THF (1.25 mL) was added at 0 °C. The mixture was stirred at room temperature under an Ar atmosphere for 2 h. A saturated aqueous solution of $NaHCO_3$ (10 mL) was added, and the whole was extracted three times with CH_2Cl_2 (10 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated to afford a colorless oil. The residue was column-chromatographed on an open column with silica gel (*n*-hexane/AcOEt/Et_3N = 40:1:1 to 40:3:1) to afford **25** as a colorless oil (135.3 mg, 0.2330 mmol, 65%).

^{1}H NMR (400 MHz, $CDCl_3$): 7.343−7.240 (15H, m), 5.214−4.987 and 4.829−4.801 (5H, 2m), 4.731−4.563 (2H, m), 4.147−3.952 (2H, m), 3.641−3.548 (2H, m), 3.015 and 3.010 (3H, 2s), 1.161−1.121 (12H, m) (two rotamers). ^{13}C NMR (100 MHz, $CDCl_3$): 169.28, 169.26, 169.20, 156.78, 156.72, 156.01, 155.97, 139.35, 139.28, 136.58, 136.37, 135.46, 135.30, 128.53, 128.50, 128.42, 128.38, 128.30, 128.22, 128.06, 127.91, 127.82, 127.80, 127.73, 127.72, 127.30, 127.26, 127.23, 126.92, 126.89, 126.86, 67.38, 66.85, 65.39, 65.33, 65.28, 65.22, 65.15, 65.09, 61.72, 61.55, 61.48, 61.42, 61.31, 61.26, 61.09, 60.93, 60.51, 60.43, 60.35, 60.25, 60.17, 43.13, 43.00, 32.73, 32.60, 32.46, 32.25, 24.57, 24.55, 24.51, 24.48, 24.45 (two rotamers). HRMS (ESI-TOF, $[M + Na]^+$): calcd for $C_{32}H_{41}N_2NaO_6P^+$, 603.2594; found, 603.2586.

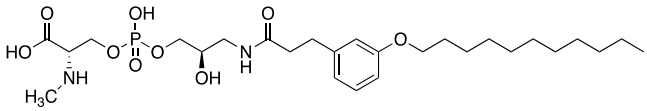
*Compound **26**.*



Compounds **25** (110.9 mg, 0.1910 mmol) and **2** (54.2 mg, 0.112 mmol) were dissolved in CH_2Cl_2 and toluene. To remove traces of water, the solution was evaporated. The mixture was dissolved in anhydrous CH_2Cl_2 (1.0 mL). A solution of 1*H*-tetrazole (16.5 mg, 0.236 mmol) in anhydrous THF (1.0 mL) was added. The mixture was stirred at room temperature under an Ar atmosphere for 3.1 h. TBHP in decane (5.0−6.0 M) (41 μL) was added, and the mixture was stirred at room temperature for 2 h. H_2O (10 mL) was added, and the whole was extracted three times with CH_2Cl_2 (10 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated to afford a colorless oil. The residue was column-chromatographed on a flash column with silica gel ($CHCl_3$/AcOEt = 4:1) to afford **26** as a colorless oil (67.0 mg, 0.0684 mmol, 61%).

^{1}H NMR (400 MHz, $CDCl_3$): (mixture of diastereomers and two rotamers) 7.305−7.114 (21H, m), 6.742−6.691 (3H, m), 6.111−5.999 (1H, m), 5.186−4.975 (6H, m), 4.889−4.829 and 4.654−4.606 (1H, 2m), 4.587−4.552 (1H, m), 4.517−4.307 (3H, m), 4.025−3.978 (1H, m), 3.906−3.848 (3H, m), 3.587−3.551 (1H, m), 3.404−3.266 (2H, m), 2.933−2.851 (5H, m), 2.392 (2H, t, J = 7.2 Hz), 1.741 (2H, quin, J = 6.9 Hz), 1.427−1.391 (2H, m), 1.299−1.264 (14H, m), 0.879 (3H, t, J = 6.7 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): (mixture of diastereomers and two rotamers) 172.33, 172.27, 167.94, 167.89, 159.21, 156.58, 156.55, 155.62, 142.29, 137.64, 136.14, 135.91, 135.37, 134.97, 134.82, 129.31, 128.66, 128.55, 128.42, 128.21, 128.18, 128.08, 128.01, 127.94, 127.91, 127.87, 127.62, 120.37, 114.62, 111.99, 75.34, 71.78, 69.67, 69.61, 67.78, 67.60, 67.56, 67.23, 67.19, 66.47, 64.74, 64.63, 64.57, 59.85, 38.88, 38.00, 33.75, 33.55, 32.90, 32.75, 31.82, 31.56, 29.53, 29.50, 29.34, 29.25, 25.99, 22.60, 14.04. HRMS (ESI-TOF, $[M + Na]^+$): calcd for $C_{56}H_{71}KN_2O_{11}P^+$, 1017.4427; found, 1017.4450.

*Compound **7c**.*



Compound **26** (60.0 mg, 0.0613 mmol) and Pd/C (8.5 mg) were dissolved in MeOH (2 mL) and AcOH (0.5 mL). The mixture was stirred at room temperature under a H_2 atmosphere for 27.4 h. The mixture was filtered on Celite, and the solvent was evaporated. The residue was purified by reversed-phase medium-pressure liquid chromatography (CH_3CN/H_2O = 3:7 to 7:3, containing 0.1% HCOOH). The solid was vacuum-dried at 40 °C to afford **7c** as a colorless powder (14.4 mg, 0.0251 mmol, 41%).

^{1}H NMR (400 MHz, DMSO-d_6): 7.938 (1H, t, J = 5.7 Hz), 7.148 (1H, dd, J = 8.1 Hz, 8.1 Hz), 6.756−6.698 (3H, m), 4.177 (1H, t, J = 11.0 Hz), 4.073−3.994 (1H, m), 3.928−3.879 (3H, m), 3.623−3.529 (4H, m), 3.174−3.112 (1H, m), 3.046−2.984 (1H, m), 2.755 (2H, t, J

https://doi.org/10.1021/acs.jmedchem.1c00347
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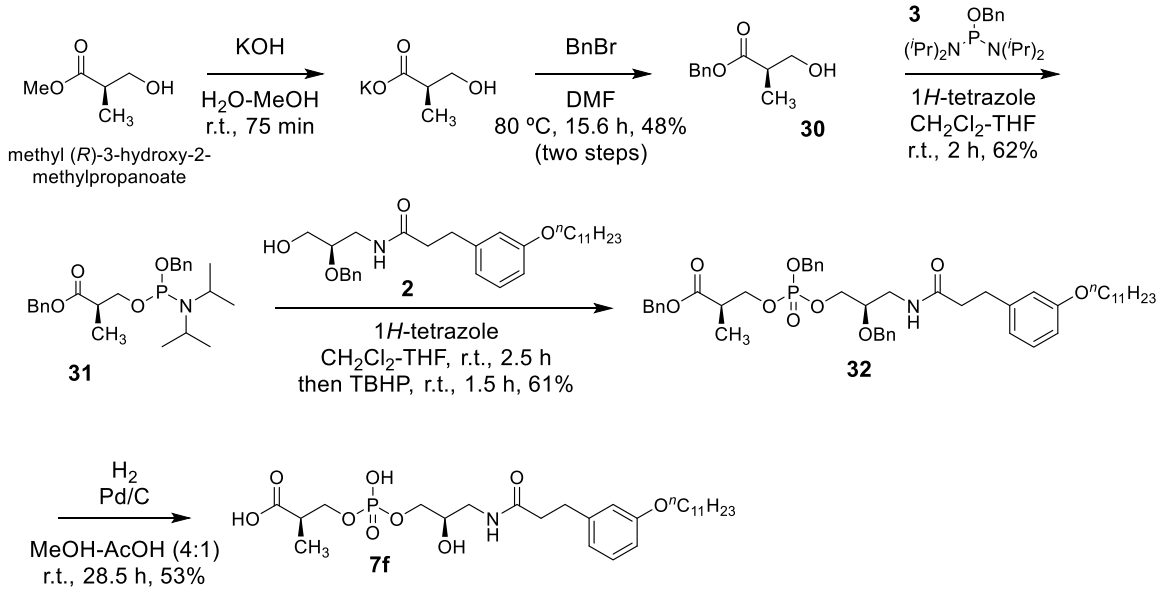
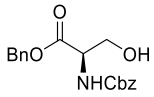


Figure 11. Synthesis of **7f**.

= 7.9 Hz), 2.558 (3H, s), 2.388−2.349 (2H, m), 1.684 (2H, quin, J = 6.9 Hz), 1.408−1.358 (2H, m), 1.249 (14H, br s), 0.855 (3H, t, J = 6.9 Hz). HRMS (ESI-TOF, $[M - H]^-$): calcd for $C_{27}H_{46}N_2O_9P^-$, 573.2946; found, 573.2948. Anal. Calcd for $C_{27}H_{47}N_2O_9P{\cdot}2H_2O$: C, 53.10; H, 8.42; N, 4.59. Found: C, 53.28; H, 8.16; N, 4.81. HPLC: 98% (CH_3CN/H_2O = 1:1 (0.1% HCO_2H)).

Synthesis of 7e (Figure 10). *Compounds **27** and **28**.*



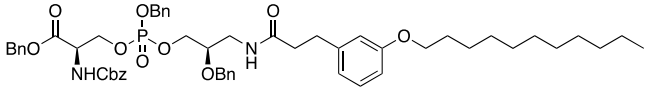
D-Serine (1051.2 mg, 10.003 mmol) was dissolved in THF (20 mL) and a saturated aqueous solution of $NaHCO_3$ (15 mL). *Z*-Cl (2050.1 mg, 12.018 mmol) and THF (10 mL) were added, and the whole was stirred at room temperature under an Ar atmosphere for 24.2 h. H_2O (15 mL) and 2 M aqueous solution of HCl (15 mL) were added, and the whole was extracted three times with AcOEt (30 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated to afford a pale yellow solid. The residue was column-chromatographed on an open column with silica gel (*n*-hexane/acetone = 2:1 to 0:1) to afford **27** (1534.2 mg) (Figure 10).

Compound **27** (1521.8 mg) and TBAI (702.9 mg, 1.908 mmol) were dissolved in anhydrous THF (40 mL). Et_3N (2.65 mL) and BnBr (905 μL) were added at 0 °C. Then, the mixture was stirred at room temperature under an Ar atmosphere for 18.4 h. H_2O (40 mL) was added, and the whole was extracted three times with AcOEt (40 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated to afford a pale yellow oil. The residue was column-chromatographed on an open column with silica gel (*n*-hexane/AcOEt = 3:2) to afford **28** as a colorless solid (907.0 mg, 2.754 mmol, 43% from D-serine).

^{1}H NMR (400 MHz, $CDCl_3$): 7.346 (10H, br s), 5.741−5.726 (1H, m), 5.210 (2H, s), 5.117−5.114 (2H, m), 4.487 (1H, br s), 3.997 (1H, br s), 3.939 (1H, br s), 2.180−2.165 (1H, m). ^{13}C NMR (100 MHz, $CDCl_3$): 170.33, 156.24, 135.99, 135.06, 128.62, 128.52, 128.50, 128.22, 128.16, 128.09, 67.49, 67.20, 63.27, 56.15.

HRMS (ESI-TOF, $[M + Na]^+$): calcd for $C_{18}H_{19}NNaO_5^+$, 352.1155; found, 352.1163. Anal. Calcd for $C_{18}H_{19}NO_5$: C, 65.64; H, 5.82; N, 4.25. Found: C, 65.47; H, 5.90; N, 4.28.

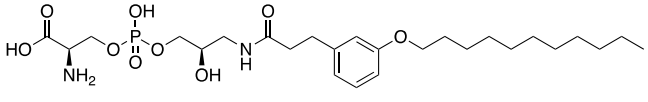
*Compound **29**.*



Compounds **28** (50.5 mg, 0.153 mmol) and **4** (161.1 mg, 0.2234 mmol) were dissolved in CH_2Cl_2 and toluene. To remove traces of water, the solution was evaporated. The mixture was dissolved in anhydrous CH_2Cl_2 (0.75 mL). A solution of 1*H*-tetrazole (21.2 mg, 0.303 mmol) in anhydrous THF (0.75 mL) was added. The mixture was stirred at room temperature under an Ar atmosphere for 2 h. TBHP in decane (5.0−6.0 M) (55 μL) was added, and the mixture was stirred at room temperature for 2 h. H_2O (10 mL) was added, and the whole was extracted three times with CH_2Cl_2 (10 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated to afford a colorless oil. The residue was column-chromatographed on a flash column with silica gel three times (first, $CHCl_3$/AcOEt = 5:1, second, $CHCl_3$/AcOEt = 6:1, third, $CHCl_3$/AcOEt = 6:1) to afford **29** as a colorless oil (pure fraction, 25.6 mg, 0.0265 mmol, 17%).

^{1}H NMR (400 MHz, $CDCl_3$): (mixtures of diastereomers) 7.318−7.257 (20H, m), 7.159−7.119 (1H, m), 6.733−6.695 (3H, m), 6.141 (J = 8.2 Hz) and 5.940 (J = 8.3 Hz) (1H, 2d), 5.882−5.833 (1H, m), 5.208−4.954 (6H, m), 4.598−4.542 (2H, m), 4.473−4.360 (2H, m), 4.319−4.239 (1H, m), 4.064−3.850 (4H, m), 3.574−3.551 (1H, m), 3.407−3.240 (2H, m), 2.874−2.818 (2H, m), 2.378−2.296 (2H, m), 1.743 (2H, quin, J = 7.0 Hz), 1.475−1.391 (2H, m), 1.300−1.201 (14H, m), 0.880 (3H, t, J = 6.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): (mixtures of diastereomers) 172.25, 168.90, 168.81, 159.26, 155.90, 155.84, 142.33, 137.63, 137.47, 136.01, 135.37, 135.31, 134.89, 129.37, 128.74, 128.63, 128.60, 128.49, 128.26, 128.21, 128.08, 127.98, 127.96, 120.41, 114.66, 112.04, 75.48, 75.42, 71.91, 71.73, 69.82, 69.77, 67.84, 67.74, 67.68, 67.43, 67.17, 66.67, 66.61, 54.57, 54.51, 54.44, 39.05, 38.07, 37.98, 31.87, 31.56, 29.58, 29.55, 29.40, 29.30, 26.04, 22.65, 14.09. HRMS (ESI-TOF, $[M + Na]^+$): calcd for $C_{55}H_{69}N_2NaO_{11}P^+$, 987.4531; found, 987.4503.

*Compound **7e**.*



https://doi.org/10.1021/acs.jmedchem.1c00347
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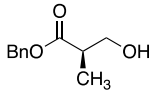
Compound **29** (23.4 mg, 0.0242 mmol) and Pd/C (10.2 mg) were dissolved in MeOH (3.0 mL). The mixture was stirred at room temperature under a H_2 atmosphere for 22 h. The mixture was filtered on Celite, and the solvent was evaporated. The residue was purified by reversed-phase medium-pressure liquid chromatography (CH_3CN/H_2O = 1:3 to 7:3, containing 0.1% HCOOH) to afford **7e** (4.6 mg, 0.0082 mmol, 34%). ^{1}H NMR (400 MHz, DMSO-d_6): 8.670 (2H, br s), 7.950 (1H, t, J = 5.7 Hz), 7.148 (1H, dd, J = 8.1 Hz, 8.1 Hz), 6.756−6.699 (3H, m), 4.058−4.019 (2H, m), 3.929−3.897 (3H, m), 3.593−3.542 (3H, m), 3.138−2.993 (2H, m), 2.755 (2H, t, J = 7.8 Hz), 2.366 (2H, t, J = 8.0 Hz), 1.685 (2H, quin, J = 6.9 Hz), 1.394−1.250 (16H, m), 0.856 (3H, t, J = 6.8 Hz). HRMS (ESI-TOF, [M − H]$^-$): calcd for $C_{26}H_{44}N_2O_9P^-$, 559.2790; found, 559.2776. HPLC: 98% (CH_3CN/H_2O = 1:1 (0.1% HCO_2H)).

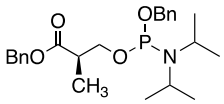
Synthesis of 7f (Figure 11). *Compound **30**.*



Methyl (*R*)-(−)-3-hydroxyisobutyrate (243.3 mg, 2.060 mmol) was dissolved in MeOH (2 mL). An aqueous solution of KOH (1.95 M, 2 mL) was added, and the whole was stirred at room temperature for 75 min. The solvent was evaporated. The residue was dissolved in anhydrous DMF (3 mL), and benzyl bromide (245 μL) was added to the mixture. The mixture was heated to 80 °C under an Ar atmosphere for 15.6 h. A 2 M aqueous solution of HCl (5 mL) and H_2O (10 mL) were added, and the whole was extracted three times with AcOEt (10 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered, and the solvent was evaporated. The residue was column-chromatographed on a flash column with silica gel (*n*-hexane/AcOEt = 3:1) to afford **30** as a colorless oil (192.1 mg, 0.9890 mmol, 48%) (Figure 11).

^{1}H NMR (400 MHz, $CDCl_3$): 7.394−7.304 (5H, m), 5.519 (2H, s), 3.777−3.692 (2H, m), 2.771−2.687 (1H, m), 1.202 (3H, d, J = 7.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): 175.44, 135.76, 128.59, 128.28, 128.03, 66.40, 64.53, 41.75, 13.40. HRMS (ESI-TOF, [M + Na]$^+$): calcd for $C_{11}H_{14}NaO_3^+$, 217.0835; found, 217.0840.

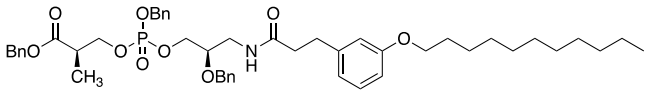
*Compound **31**.*



Compound **30** (183.8 mg, 0.9470 mmol) was dissolved in CH_2Cl_2 and toluene. To remove traces of water, the solution was evaporated. Compound **3** (676.1 mg, 1.997 mmol) was added, and the mixture was dissolved in anhydrous CH_2Cl_2 (3.0 mL). A solution of 1*H*-tetrazole (67.5 mg, 0.964 mmol) in anhydrous THF (3.0 mL) was added at 0 °C. The mixture was stirred at room temperature under an Ar atmosphere for 2 h. A saturated aqueous solution of $NaHCO_3$ (10 mL) was added, and the aqueous layer was extracted three times with CH_2Cl_2 (10 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated to afford a colorless oil. The residue was column-chromatographed on an open column with silica gel (first, *n*-hexane/AcOEt/Et_3N = 40:2:1, second *n*-hexane/AcOEt/Et_3N = 40:1:1) to afford **31** as a colorless oil (252.5 mg, 0.5852 mmol, 62%).

^{1}H NMR (400 MHz, CD_2Cl_2): 7.348−7.229 (10H, m), 5.133−5.052 (2H, m), 4.703−4.647 (1H, m), 4.632−4.580 (1H, m), 3.895−3.552 (4H, m), 2.833−2.742 (1H, m), 1.192−1.146 (15H, m). HRMS (ESI-TOF, [M + H]$^+$): calcd for $C_{24}H_{35}NO_4P^+$, 432.2298; found, 432.2305.

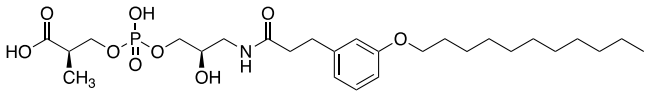
*Compound **32**.*



Compounds **31** (106.0 mg, 0.2456 mmol) and **2** (84.3 mg, 0.174 mmol) were dissolved in CH_2Cl_2 and toluene. To remove traces of water, the solution was evaporated. The residue was dissolved in anhydrous CH_2Cl_2 (1.5 mL) and a solution of 1*H*-tetrazole (24.7 mg, 0.353 mmol) in anhydrous THF (1.5 mL) was added. The mixture was stirred at room temperature under an Ar atmosphere for 2.5 h. TBHP in decane (5.0−6.0 M) (60 μL) was added, and the mixture was stirred at room temperature for 1.5 h. H_2O (10 mL) was added, and the whole was extracted three times with CH_2Cl_2 (10 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated to afford a colorless oil. The residue was column-chromatographed on a flash column with silica gel twice ($CHCl_3$/AcOEt = 4:1) to afford **32** as a colorless oil (123.4 mg, 0.1487 mmol, 61%).

^{1}H NMR (400 MHz, $CDCl_3$): (mixtures of diastereomers) 7.350−7.278 (15H, m), 7.173−7.125 (1H, m), 6.749−6.698 (3H, m), 6.033−5.946 (1H, m), 5.142−5.074 (2H, m), 5.025−4.997 (2H, m), 4.590 (1H, d, J = 11.7 Hz), 4.478 (1H, d, J = 11.7 Hz), 4.232−4.165 (1H, m), 4.097−3.973 (2H, m), 3.935−3.881 (3H, m), 2.894−2.783 (3H, m), 2.407−2.368 (2H, m), 1.780−1.726 (2H, m), 1.693 (2H, br s), 1.464−1.264 (16H, m), 1.183−1.160 (3H, m), 0.880 (3H, t, J = 6.9 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): (mixtures of diastereomers) 173.19, 173.15, 172.27, 172.25, 159.26, 142.36, 137.74, 135.61, 129.37, 128.65, 128.60, 128.55, 128.48, 128.26, 128.05, 127.99, 127.97, 127.95, 120.42, 114.64, 112.05, 75.45, 75.39, 71.86, 69.55, 69.50, 68.74, 68.67, 67.84, 66.60, 66.56, 66.36, 40.45, 40.41, 40.37, 40.33, 38.99, 38.13, 31.88, 31.62, 29.59, 29.56, 29.40, 29.30, 26.05, 22.66, 14.09, 13.38. HRMS (ESI-TOF, [M + Na]$^+$): calcd for $C_{48}H_{64}NNaO_9P^+$, 852.4211; found, 852.4205. Anal. Calcd for $C_{48}H_{64}NO_9P{\cdot}0.5H_2O$: C, 68.71; H, 7.81; N, 1.67. Found: C, 68.81; H, 7.79; N, 1.58.

*Compound **7f**.*



Compound **32** (51.2 mg, 0.0617 mmol) and Pd/C (5.8 mg) were dissolved in MeOH (4 mL) and AcOH (1 mL). The mixture was stirred at room temperature under a H_2 atmosphere for 28.5 h. The mixture was filtered on Celite, and the solvent was evaporated. The residue was purified by reversed-phase medium-pressure liquid chromatography (CH_3CN/H_2O = 3:7 to 7:3, containing 0.1% HCOOH). After vacuum drying at 40 °C, the solid was washed with CH_3CN (300 μL × 3). The solid was vacuum-dried at 40 °C to afford **7f** (18.2 mg, 0.0325 mmol, 53%).

^{1}H NMR (400 MHz, DMSO-d_6): 7.889 (1H, t, J = 5.8 Hz), 7.149 (1H, dd, J = 8.1 Hz, 8.1 Hz), 6.754−6.699 (3H, m), 3.980−3.834 (4H, m), 3.752−3.608 (3H, m), 3.183−3.121 (1H, m), 3.055−2.992 (1H, m), 2.757 (2H, t, J = 7.9 Hz), 2.692−2.642 (1H, m), 2.374 (2H, t, J = 7.9 Hz), 1.684 (2H, quin, J = 6.9 Hz), 1.407−1.248 (16H, m), 1.072 (3H, d, J = 7.1 Hz), 0.855 (3H, t, J = 6.8 Hz). HRMS (ESI-TOF, [M − H]$^-$): calcd for $C_{27}H_{45}NO_9P^-$, 558.2837; found, 558.2862. Anal. Calcd for $C_{27}H_{46}NO_9P{\cdot}H_2O$: C, 56.14; H, 8.38; N, 2.42. Found: C, 56.20; H, 8.15; N, 2.35. HPLC: 95% (CH_3CN/H_2O = 3:2 (0.1% HCO_2H)).

https://doi.org/10.1021/acs.jmedchem.1c00347
J. Med. Chem. 2021, 64, 10059−10101

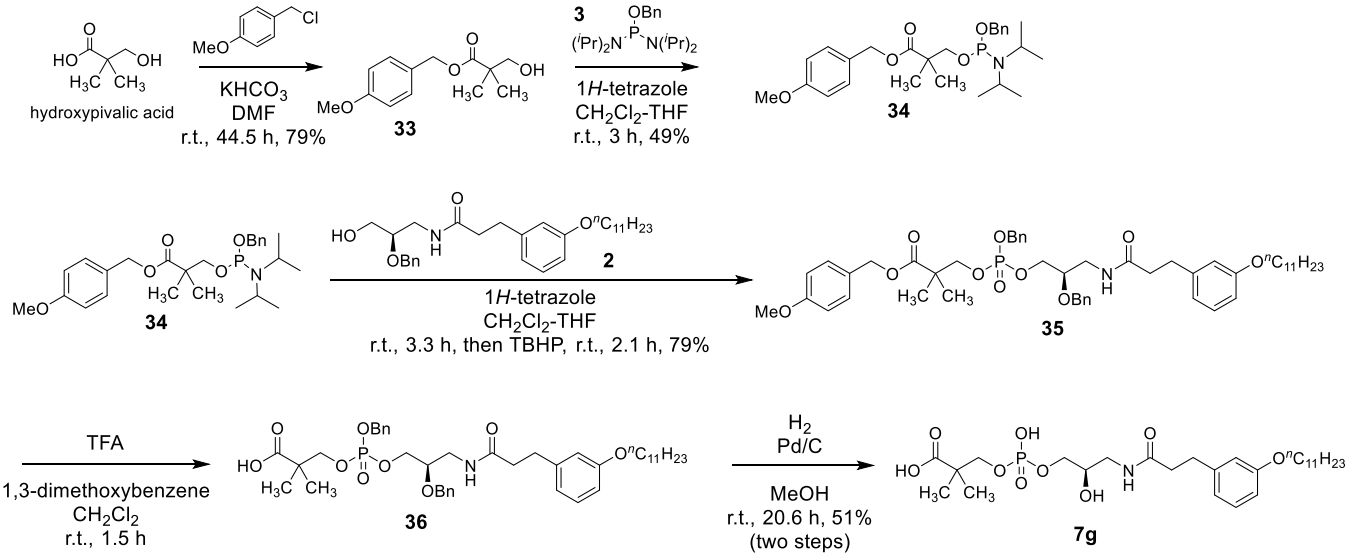
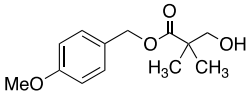


Figure 12. Synthesis of **7g**.

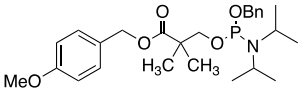
Synthesis of 7g (Figure 12). *Compound **33**.*



Hydroxypivalic acid (1000.0 mg, 8.4651 mmol), *p*-methoxybenzoyl chloride (1590.9 mg, 10.158 mmol), and $KHCO_3$ (1016.9 mg, 10.157 mmol) were dissolved in anhydrous DMF (10 mL). The mixture was stirred at room temperature for 44.5 h. H_2O (10 mL) was added, and the whole was extracted with AcOEt. The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated. The residue was column-chromatographed on an open column with silica gel (*n*-hexane/AcOE = 3:1 to 1:1) to afford **33** as a colorless oil (1590.2 mg, 6.6736 mmol, 79%) (Figure 12).

^{1}H NMR (400 MHz, $CDCl_3$): 7.272 (2H, d, J = 9.4 Hz), 6.889 (2H, d, J = 8.8 Hz), 5.078 (2H, s), 3.811 (3H, s), 3.556 (2H, s), 2.386 (1H, br s), 1.195 (6H, s). ^{13}C NMR (100 MHz, $CDCl_3$): 177.43, 159.57, 129.66, 128.62, 127.99, 113.95, 69.66, 66.20, 55.24, 44.20, 22.03. HRMS (ESI-TOF, $[M + H]^+$): calcd for $C_{13}H_{18}NaO_4^+$, 261.1097; found, 261.1085.

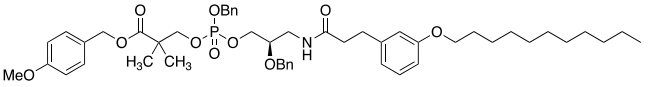
*Compound **34**.*



Compound **33** (237.8 mg, 0.9980 mmol) was dissolved in CH_2Cl_2 and toluene. To remove traces of water, the solution was evaporated. Compound **3** (517.6 mg, 1.529 mmol) and anhydrous CH_2Cl_2 (3.0 mL) were added. A solution of 1*H*-tetrazole (70.8 mg, 1.01 mmol) in anhydrous THF (3.0 mL) was added at room temperature. The mixture was stirred at room temperature under an Ar atmosphere for 3 h. A saturated aqueous solution of $NaHCO_3$ (10 mL) was added, and the whole was extracted three times with CH_2Cl_2 (10 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated to afford a colorless oil. The residue was column-chromatographed on an open column with silica gel (*n*-hexane/AcOEt/Et_3N = 40:1:1) to afford **34** as a colorless oil (232.8 mg, 0.4895 mmol, 49%).

^{1}H NMR (400 MHz, $CDCl_3$): 7.330−7.295 (4H, m), 7.275−7.246 (3H, m), 6.838 (2H, d, J = 8.8 Hz), 5.065−4.998 (2H, m), 4.695 (1H, dd, J = 12.7 Hz, 8.0 Hz), 4.618 (1H, dd, J = 12.7 Hz, 8.2 Hz), 3.777 (3H, s), 3.698 (1H, dd, J = 9.6 Hz, 5.8 Hz), 3.646−3.553 (3H, m), 1.206 (6H, d, J = 1.8 Hz), 1.165 (6H, d, J = 1.8 Hz), 1.148 (6H, d, J = 1.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): 176.12, 159.33, 139.66, 139.58, 129.55, 128.43, 128.16, 127.10, 126.82, 113.76, 70.35, 70.19, 65.95, 65.11, 64.94, 55.19, 44.27, 44.19, 43.06, 42.94, 24.59, 24.53, 24.52, 24.47, 22.17. HRMS (ESI-TOF, $[M + H]^+$): calcd for $C_{26}H_{39}NO_5P^+$, 476.2560; found, 476.2587.

*Compound **35**.*



Compounds **34** (129.6 mg, 0.2725 mmol) and **2** (81.7 mg, 0.169 mmol) were dissolved in CH_2Cl_2 and toluene. To remove traces of water, the solution was evaporated. The mixture was dissolved in anhydrous CH_2Cl_2 (1.2 mL). A solution of 1*H*-tetrazole (23.7 mg, 0.338 mmol) in anhydrous THF (1.2 mL) was added at 0 °C. The mixture was stirred at room temperature under an Ar atmosphere for 3.3 h. TBHP in decane (5.0−6.0 M) (60 μL) was added, and the mixture was stirred at room temperature for 2.1 h. H_2O (10 mL) was added, and the whole was extracted three times with CH_2Cl_2 (10 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated to afford a colorless oil. The residue was column-chromatographed on a flash column with silica gel ($CHCl_3$/AcOEt = 4:1) to afford **35** as a colorless oil (116.3 mg, 0.1331 mmol, 79%).

^{1}H NMR (400 MHz, $CDCl_3$): (mixtures of diastereomers) 7.346−7.221 (12H, m), 7.173−7.124 (1H, m), 6.830 (2H, d, J = 8.4 Hz), 6.750−6.697 (3H, m), 6.057 and 6.003 (1H, 2t, J = 5.9 Hz), 5.027 (2H, s), 5.009−4.969 (2H, m), 4.590 (1H, d, J = 11.6 Hz), 4.483 (1H, dd, J = 11.7 Hz, 2.2 Hz), 4.025−3.956 (3H, m), 3.934−3.880 (3H, m), 3.750 (3H, s), 3.606−3.569 (1H, m), 3.426−3.310 (2H, m), 2.877 (2H, t, J = 7.8 Hz), 2.414−2.371 (2H, m), 1.783−1.709 (2H, m), 1.447−1.264 (16H, m), 1.184−1.173 (6H, m), 0.880 (3H, t, J = 6.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): (mixtures of diastereomers) 174.99, 174.94, 172.27, 172.24, 159.49, 159.23, 142.35, 137.74, 135.63, 135.60, 135.57, 135.53, 129.70, 129.35, 128.60, 128.58, 128.45, 127.94, 127.92, 127.89, 120.40, 114.62, 113.85, 112.03, 75.48, 75.45, 75.41, 75.38, 73.26, 73.20, 71.82, 69.49, 69.45, 69.43, 69.40, 67.82, 66.42, 66.40, 66.34, 66.28, 55.17, 43.52, 43.50, 43.44, 43.41, 38.92, 38.10, 31.86, 31.61, 29.56, 29.54, 29.38, 29.28, 26.02, 22.63, 21.76, 14.07. HRMS (ESI-TOF, $[M + Na]^+$): calcd for $C_{50}H_{68}NNaO_{10}P^+$, 896.4473; found, 896.4480.

https://doi.org/10.1021/acs.jmedchem.1c00347
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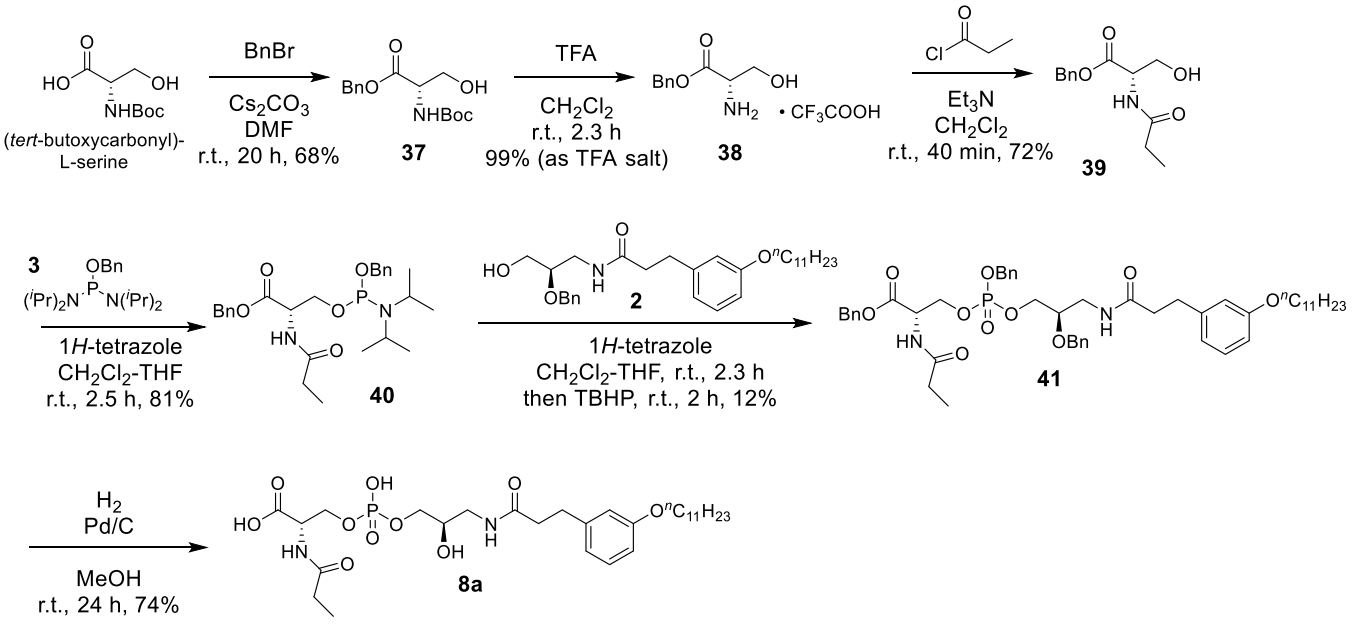
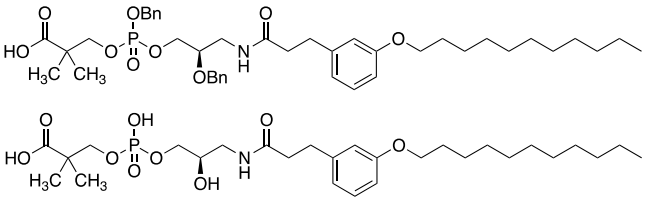


Figure 13. Synthesis of **8a**.

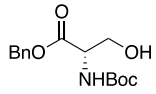
*Compounds **36** and **7g**.*



Compounds **35** (36.3 mg, 0.0415 mmol) and 1,3-dimethoxybenzene (58.1 mg, 0.421 mmol) were dissolved in anhydrous CH_2Cl_2 (0.5 mL). TFA (0.1 mL) was added at 0 °C, and the mixture was stirred at room temperature for 1.5 h. CH_2Cl_2 (ca. 2 mL) was added, and the solvent was removed by evaporation. The residue (crude **36**, colorless oil, 105.2 mg) and Pd/C (18.1 mg) were dissolved in MeOH (2.0 mL). The mixture was stirred at room temperature under a H_2 atmosphere for 20.6 h. Then, the mixture was filtered on Celite, and the solvent was evaporated. The residue was purified by a flash column with silica gel ($CHCl_3$/MeOH/AcOH = 8:1:1) to afford **7g** as a pale yellow powder (12.1 mg, 0.0211 mmol, 51%).

^{1}H NMR (400 MHz, DMSO-d_6): 8.013 (1H, br s), 7.140 (1H, dd, J = 8.1 Hz, 8.1 Hz), 6.752−6.690 (3H, m), 3.909 (2H, t, J = 6.5 Hz), 3.585−3.506 (4H, m), 3.043 (2H, br s), 2.750 (2H, t, J = 7.9 Hz), 2.357 (2H, t, J = 8.0 Hz), 1.681 (2H, quin, J = 6.9 Hz), 1.405−1.247 (16H, m), 1.025 (6H, s), 0.853 (3H, t, J = 6.8 Hz). HRMS (ESI-TOF, [M − H]$^-$): calcd for $C_{28}H_{47}NO_9P^-$, 572.2994; found, 572.2993. HPLC: 99% (CH_3CN/H_2O = 3:2 (0.1% HCO_2H)).

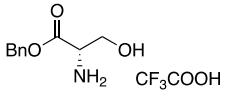
Synthesis of 8a (Figure 13). *Compound **37**.*



(*tert*-Butoxycarbonyl)-L-serine (1443.2 mg, 7.0328 mmol) and Cs_2CO_3 (2741.1 mg, 8.4130 mmol) were dissolved in anhydrous DMF (40 mL). Benzyl bromide (1.0 mL) was added, and the mixture was stirred at room temperature under an Ar atmosphere for 20 h. H_2O (70 mL) was added, and the whole was extracted three times with Et_2O (40 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated. The residue was column-chromatographed on an open column with silica gel twice (first, *n*-hexane/AcOEt = 1:1, second, *n*-hexane/AcOEt = 2:1) to afford **37** as a colorless oil (1408.4 mg, 4.7688 mmol, 68%) (Figure 13).

^{1}H NMR (400 MHz, $CDCl_3$): 7.372−7.328 (5H, m), 5.456 (1H, br s), 5.247−5.180 (2H, m), 4.421 (1H, br s), 3.983 (1H, dd, J = 11.2 Hz, 3.8 Hz), 3.910 (1H, dd, J = 11.2 Hz, 3.4 Hz), 1.440 (9H, s). ^{13}C NMR (100 MHz, $CDCl_3$): 170.64, 155.71, 135.18, 128.62, 128.48, 128.17, 80.33, 67.39, 63.62, 55.84, 28.26. HRMS (ESI-TOF, [M + Na]$^+$): calcd for $C_{15}H_{21}NNaO_5^+$, 318.1312; found, 318.1304.

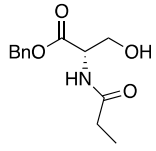
*Compound **38**.*



To the solution of **37** (1374.7 mg, 4.6547 mmol) in CH_2Cl_2 (17.5 mL), TFA (3.5 mL) was added at 0 °C. The mixture was stirred at room temperature for 2.3 h. The solvent was evaporated, and the residue was dried to afford **38** as a pale yellow solid (1426.5 mg, 4.6129 mmol, 99%, calculated as TFA salt), which was used without further purification.

HRMS (ESI-TOF, [M + H]$^+$): calcd for $C_{10}H_{14}NO_3^+$, 196.0968; found, 196.0952.

*Compound **39**.*



Compound **38** (620.0 mg, 2.005 mmol) was dissolved in anhydrous CH_2Cl_2 (8.0 mL). Et_3N (560 μL) and propionyl chloride (0.2 mL, 2.3 mmol) were added, and the mixture was stirred at room temperature under an Ar atmosphere for 40 min. MeOH (ca. 2 mL) was added, and the solvent was evaporated with silica gel. The residue was column-chromatographed on an open column with silica gel (*n*-hexane/acetone = 3:2) to afford **39** as a pale yellow solid (364.4 mg, 1.450 mmol, 72%).

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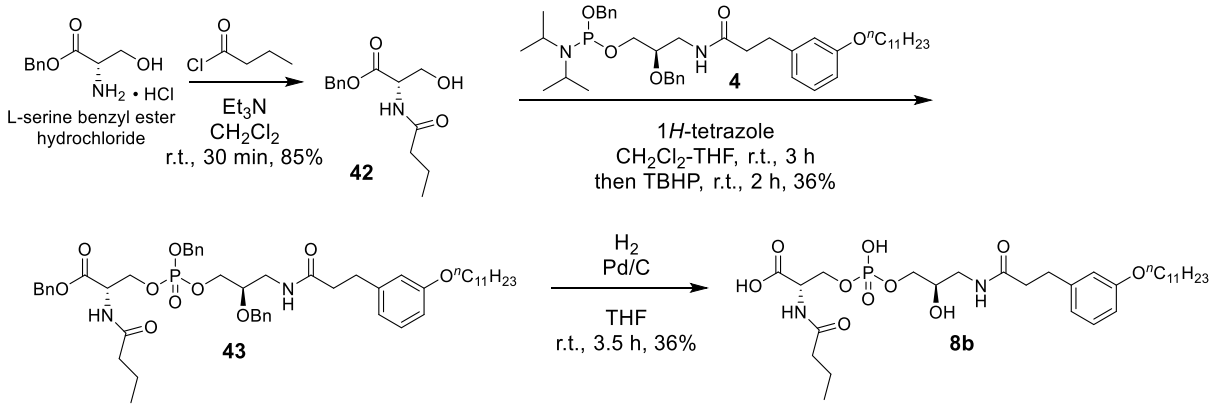
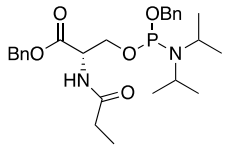


Figure 14. Synthesis of **8b**.

^{1}H NMR (400 MHz, $CDCl_3$): 7.394−7.318 (5H, m), 6.476 (1H, d, J = 6.6 Hz), 5.246−5.182 (2H, m), 4.735−4.698 (1H, m), 3.990 (1H, dd, J = 11.2 Hz, 4.0 Hz), 3.923 (1H, dd, J = 11.2 Hz, 3.4 Hz), 2.518 (1H, br s), 2.294 (2H, q, J = 7.6 Hz), 1.165 (3H, t, J = 7.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): 174.39, 170.41, 135.05, 128.65, 128.54, 128.15, 67.53, 63.57, 54.78, 29.44, 9.55. HRMS (ESI-TOF, $[M + Na]^+$): calcd for $C_{13}H_{17}NNaO_4^+$, 274.1050; found, 274.1060. mp 51.5−53.0 °C (colorless needles, recrystallized from n-hexane/CH_2Cl_2).

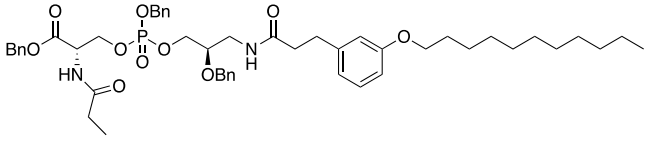
*Compound **40**.*



Compound **39** (252.3 mg, 1.004 mmol) was dissolved in CH_2Cl_2 and toluene. To remove traces of water, the solution was evaporated. Compound **3** (crude, 571.6 mg, 1.689 mmol) was added, and the mixture was dissolved in anhydrous CH_2Cl_2 (4.0 mL). A solution of 1*H*-tetrazole (72.9 mg, 1.04 mmol) in anhydrous THF (4.0 mL) was added at 0 °C. The mixture was stirred at room temperature under an Ar atmosphere for 2.5 h. A saturated aqueous solution of $NaHCO_3$ (10 mL) was added, and the whole was extracted three times with CH_2Cl_2 (15 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated to afford a colorless oil. The residue was column-chromatographed on an open column with silica gel (n-hexane/AcOEt/Et_3N = 40:1:1 to 40:16:1) to afford **40** as a colorless oil (394.9 mg, 0.8083 mmol, 81%).

^{1}H NMR (400 MHz, $CDCl_3$): 7.380−7.272 (10H, m), 6.552 (J = 8.1 Hz) and 6.303 (J = 8.0 Hz) (1H, 2d), 5.170−5.146 (2H, m), 4.813−4.555 (3H, m), 4.226−4.112 (1H, m), 3.924−3.821 (1H, m), 3.635−3.533 (2H, m), 2.244−2.185 (1H, m), 1.996−1.870 (1H, m), 1.267−1118 (13.5H, m), 1.105 (1.5H, t, J = 7.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): 173.65, 173.47, 170.08, 169.99, 139.20, 139.13, 138.83, 135.46, 135.29, 128.53, 128.51, 128.45, 128.32, 128.29, 128.25, 128.19, 128.03, 127.63, 127.37, 127.27, 126.97, 67.26, 67.12, 65.51, 65.33, 65.27, 65.10, 64.40, 64.25, 63.64, 63.49, 53.47, 53.42, 53.34, 43.20, 43.12, 43.07, 43.00, 29.47, 29.15, 24.65, 24.60, 24.58, 24.53, 24.46, 9.56. HRMS (ESI-TOF, $[M + Na]^+$): calcd for $C_{26}H_{37}N_2NaO_5P^+$, 511.2332; found, 511.2337.

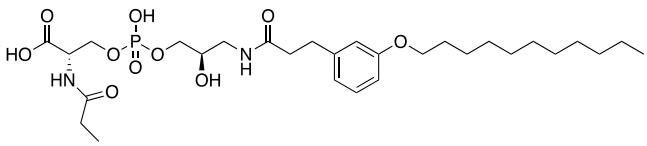
*Compound **41**.*



Compounds **40** (383.2 mg, 0.7843 mmol) and **2** (223.8 mg, 0.4627 mmol) were dissolved in CH_2Cl_2 and toluene. To remove traces of water, the solution was evaporated. The mixture was dissolved in anhydrous CH_2Cl_2 (4.0 mL). A solution of 1*H*-tetrazole (63.6 mg, 0.908 mmol) in anhydrous THF (4.0 mL) was added at 0 °C. The mixture was stirred at room temperature under an Ar atmosphere for 2.3 h. TBHP in decane (5.0−6.0 M) (168 μL) was added, and the mixture was stirred at room temperature for 2 h. H_2O (10 mL) was added, and the whole was extracted three times with CH_2Cl_2 (10 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated to afford a colorless oil. The residue was column-chromatographed on a flash column with silica gel ($CHCl_3$/AcOEt = 3:2) to afford **41** as a colorless oil (pure fraction, 51.1 mg, 0.0576 mmol, 12%).

^{1}H NMR (400 MHz, $CDCl_3$): (mixtures of diastereomers) 7.354−7.274 (15H, m), 7.199−7.128 (1H, m), 6.811−6.660 (3H, m), 5.980−5.915 (1H, m), 5.223−5.118 (2H, m), 4.997 and 4.975 (2H, 2s), 4.831 (1H, br s), 4.611−4.566 (1H, m), 4.476−4.381 (2H, m), 4.296−4.247 (1H, m), 4.018−3.827 (4H, m), 3.597−3.573 (1H, m), 3.362−3.336 (2H, m), 2.976 (J = 7.9 Hz) and 2.877 (J = 7.7 Hz) (2H, 2t), 2.403 (2H, t, J = 6.9 Hz), 2.313−2.177 (2H, m), 1.822 (2H, br s), 1.780−1.727 (2H, m), 1.465−1.263 (16H, m), 1.176 (J = 7.6 Hz) and 1.117 (J = 7.6 Hz) (3H, 2t), 0.879 (3H, t, J = 6.7 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): (mixtures of diastereomers) 173.96, 172.38, 168.88, 159.24, 142.24, 137.57, 137.54, 135.31, 135.25, 134.93, 129.36, 128.78, 128.75, 128.64, 128.56, 128.48, 128.45, 128.21, 128.15, 128.00, 127.94, 120.37, 114.65, 112.00, 75.45, 75.39, 71.94, 69.81, 69.75, 67.82, 67.58, 67.49, 67.43, 67.38, 67.31, 66.63, 66.57, 52.65, 52.58, 39.08, 38.03, 31.83, 31.55, 29.54, 29.52, 29.36, 29.26, 29.16, 26.01, 22.61, 14.06, 9.45. HRMS (ESI-TOF, $[M + Na]^+$): calcd for $C_{50}H_{67}N_2NaO_{10}P^+$, 909.4426; found, 909.4418.

*Compound **8a**.*



Compound **41** (47.9 mg, 0.0540 mmol) and Pd/C (5.7 mg) were dissolved in MeOH (10 mL). The mixture was stirred at room temperature under a H_2 atmosphere for 24 h. The mixture was filtered on Celite, and the solvent was evaporated. The residue was purified by reversed-phase medium-pressure liquid chromatography (CH_3CN/H_2O = 3:7 to 7:3, containing 0.1% HCOOH) to afford **8a** as a colorless powder (24.5 mg, 0.0397 mmol, 74%).

^{1}H NMR (400 MHz, DMSO-d_6): 12.786 (1H, br s), 8.267 (1H, d, J = 8.0 Hz), 7.878 (1H, t, J = 5.6 Hz), 7.149 (1H, dd, J = 8.0 Hz, 8.0 Hz),

https://doi.org/10.1021/acs.jmedchem.1c00347
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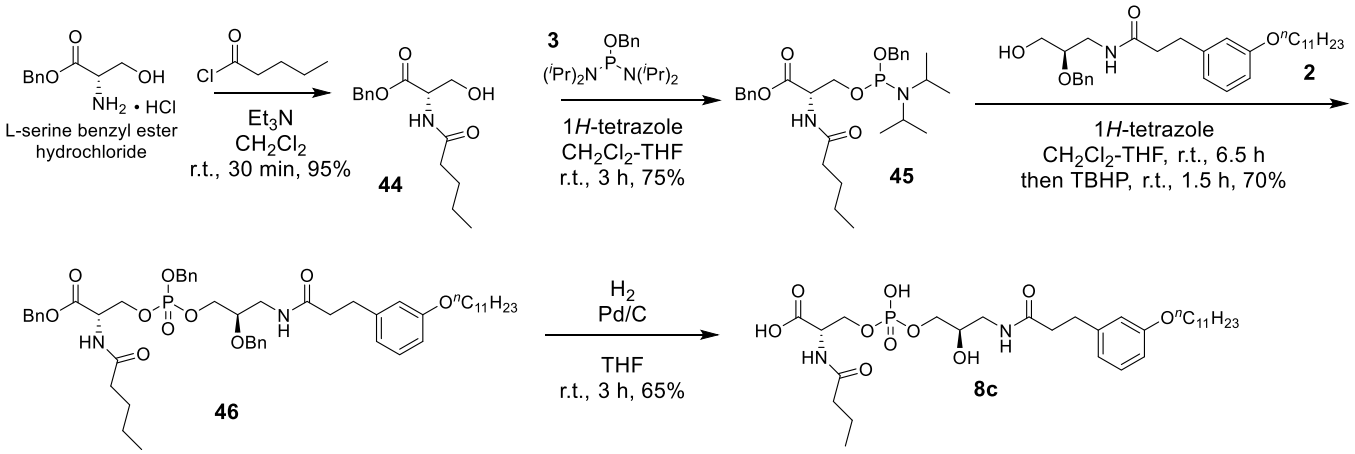
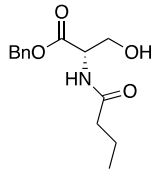


Figure 15. Synthesis of **8c**.

6.751−6.700 (3H, m), 4.491−4.449 (1H, m), 4.124−4.013 (2H, m), 3.911 (2H, t, J = 6.5 Hz), 3.758−3.614 (3H, m), 3.184−3.123 (1H, m), 3.054−2.991 (1H, m), 2.757 (2H, t, J = 7.9 Hz), 2.376 (2H, t, J = 7.9 Hz), 2.159 (2H, q, J = 7.6 Hz), 1.683 (2H, quin, J = 6.9 Hz), 1.425−1.248 (16H, m), 0.987 (3H, t, J = 7.6 Hz), 0.854 (3H, t, J = 6.8 Hz). HRMS (ESI-TOF, $[M - H]^-$): calcd for $C_{29}H_{48}N_2O_{10}P^-$, 615.3052; found, 615.3053. Anal. Calcd for $C_{20}H_{49}N_2O_{10}P·0.7H_2O$: C, 55.35; H, 8.07; N, 4.45. Found: C, 55.28; H, 7.87; N, 4.47. HPLC: 96% (CH_3CN/H_2O = 1:1 (0.1% HCO_2H)).

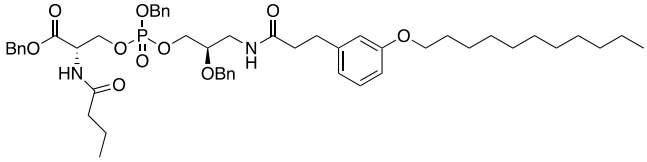
Synthesis of 8b (Figure 14). *Compound **42**.*



L-Serine benzyl ester hydrochloride (694.0 mg, 2.996 mmol) was dissolved in anhydrous CH_2Cl_2 (10 mL). Et_3N (1040 μL) and butyryl chloride (375 μL, 3.59 mmol) were added, and the mixture was stirred at room temperature under an Ar atmosphere for 30 min. MeOH (ca. 2 mL) was added, and the solvent was evaporated with silica gel. The residue was column-chromatographed on an open column with silica gel (n-hexane/acetone = 2:1 to 3:2) to afford **42** as a colorless solid (675.8 mg, 2.547 mmol, 85%) (Figure 14).

^{1}H NMR (400 MHz, $CDCl_3$): 7.396−7.320 (5H, m), 6.446 (1H, d, J = 6.8 Hz), 5.249−5.183 (2H, m), 4.745−4.708 (1H, m), 3.993 (1H, ddd, J = 11.1 Hz, 5.7 Hz, 4.0 Hz), 3.922 (1H, ddd, J = 11.1 Hz, 6.1 Hz, 3.3 Hz), 2.630 (1H, t, J = 5.9 Hz), 2.241 (2H, t, J = 7.5 Hz), 1.721−1.628 (2H, m), 0.951 (3H, t, J = 7.4 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): 173.57, 170.41, 135.06, 128.65, 128.55, 128.18, 67.53, 63.67, 54.76, 38.34, 18.96, 13.65. HRMS (ESI-TOF, $[M + Na]^+$): calcd for $C_{14}H_{19}NNaO_4^+$, 288.1206; found, 288.1217. Anal. Calcd for $C_{14}H_{19}NO_4·0.5H_2O$: C, 61.30; H, 7.35; N, 5.11. Found: C, 61.66; H, 7.20; N, 5.17. mp 41.0−42.0 °C (colorless powder, recrystallized from n-hexane/CH_2Cl_2).

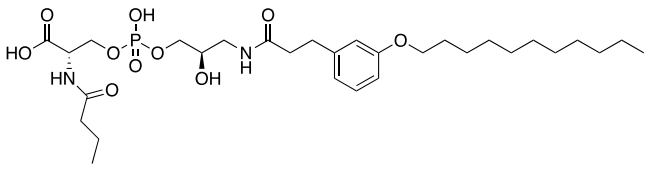
*Compound **43**.*



Compounds **42** (40.8 mg, 0.154 mmol) and **4** (173.4 mg, 0.2405 mmol) were dissolved in CH_2Cl_2 and toluene. To remove traces of water, the solution was evaporated. The mixture was dissolved in anhydrous CH_2Cl_2 (0.75 mL). A solution of 1H-tetrazole (22.1 mg, 0.315 mmol) in anhydrous THF (0.75 mL) was added at 0 °C. The mixture was stirred at room temperature under an Ar atmosphere for 3 h. TBHP in decane (5.0−6.0 M) (58 μL) was added, and the mixture was stirred at room temperature for 2 h. H_2O (10 mL) was added, and the whole was extracted three times with CH_2Cl_2 (10 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated to afford a colorless oil. The residue was column-chromatographed on a flash column with silica gel three times (first, n-hexane/acetone = 2:1, second, n-hexane/acetone = 3:1, third, n-hexane/acetone = 3:1) to afford **43** as a colorless oil (49.5 mg, 0.0549 mmol, 36%).

^{1}H NMR (400 MHz, $CDCl_3$): (mixtures of diastereomers) 7.353−7.272 (15H, m), 7.172−7.125 (1H, m), 6.852−6.699 (4H, m), 5.946 and 5.882 (1H, 2t, J = 5.9 Hz), 5.196−5.115 (2H, m), 4.996−4.974 (2H, m), 4.861−4.808 (1H, m), 4.581 (1H, d, J = 11.7 Hz), 4.464−4.381 (2H, m), 4.288−4.215 (1H, m), 4.024−3.835 (4H, m), 3.636−3.545 (1H, m), 3.365−3.329 (2H, m), 2.871 (2H, t, J = 7.8 Hz), 2.437−2.347 (2H, m), 2.204−2.135 (2H, m), 1.782−1.712 (2H, m), 1.673−1.572 (2H, m), 1.464−1.392 (2H, m), 1.347−1.263 (14H, m), 0.927−0.861 (6H, m). ^{13}C NMR (100 MHz, $CDCl_3$): (mixtures of diastereomers) 173.17, 173.14, 172.34, 172.33, 168.89, 168.86, 159.26, 142.27, 137.60, 137.57, 135.34, 135.28, 134.96, 134.95, 129.38, 128.79, 128.76, 128.66, 128.58, 128.52, 128.50, 128.48, 128.25, 128.18, 128.03, 128.01, 127.95, 120.39, 114.67, 112.03, 75.53, 75.49, 75.47, 75.42, 71.95, 69.83, 69.79, 69.73, 67.84, 67.62, 67.61, 67.52, 67.47, 67.43, 67.37, 66.65, 66.59, 66.54, 66.48, 52.63, 52.56, 39.13, 39.11, 38.07, 38.04, 31.85, 31.57, 29.56, 29.54, 29.38, 29.28, 26.02, 22.63, 18.84, 14.07, 13.64. HRMS (ESI-TOF, $[M + NH_4]^+$): calcd for $C_{51}H_{73}N_3O_{10}P^+$, 918.5028; found, 918.5020.

*Compound **8b**.*



Compound **43** (46.0 mg, 0.0510 mmol) and Pd/C (15.8 mg) were dissolved in THF (5 mL). The mixture was stirred at room temperature under a H_2 atmosphere for 3.5 h. The mixture was filtered on Celite, and the solvent was evaporated. The residue was purified by reversed-phase medium-pressure liquid chromatography (CH_3CN/H_2O = 3:7 to 7:3, containing 0.1% HCOOH) to afford **8b** as a colorless powder (11.7 mg, 0.0186 mmol, 36%).

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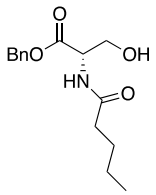
^{1}H NMR (400 MHz, DMSO-d_6): 8.284 (1H, d, J = 7.8 Hz), 7.876 (1H, t, J = 5.7 Hz), 7.149 (1H, dd, J = 8.1 Hz, 8.1 Hz), 6.754−6.700 (3H, m), 4.495−4.453 (1H, m), 4.123−4.017 (2H, m), 3.911 (2H, t, J = 6.5 Hz), 3.772−3.601 (4H, m), 3.185−3.123 (1H, m), 3.055−2.991 (1H, m), 2.758 (2H, t, J = 7.9 Hz), 2.377 (2H, t, J = 7.9 Hz), 2.124 (2H, t, J = 7.3 Hz), 1.683 (2H, quin, J = 6.9 Hz), 1.509 (2H, sextet, J = 7.4 Hz), 1.407−1.356 (2H, m), 1.248 (14H, br s), 0.872−0.835 (6H, m). HRMS (ESI-TOF, [M − H]$^-$): calcd for $C_{30}H_{50}N_2O_{10}P^-$, 629.3209; found, 629.3228. HPLC: >99% (CH_3CN/H_2O = 1:1 (0.1% HCO_2H)).

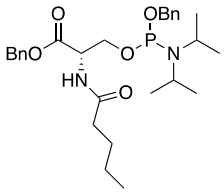
Synthesis of 8c (Figure 15). *Compound **44**.*



L-Serine benzyl ester hydrochloride (693.6 mg, 2.994 mmol) was dissolved in anhydrous CH_2Cl_2 (10 mL). Et_3N (1040 μL) and valeryl chloride (400 μL, 3.32 mmol) were added, and the mixture was stirred at room temperature under an Ar atmosphere for 30 min. MeOH (ca. 2 mL) was added, and the solvent was evaporated with silica gel. The residue was column-chromatographed on an open column with silica gel (*n*-hexane/acetone = 2:1 to 3:2) to afford **44** as a colorless solid (794.5 mg, 2.844 mmol, 95%) (Figure 15).

^{1}H NMR (400 MHz, $CDCl_3$): 7.400−7.314 (5H, m), 6.436 (1H, d, J = 6.8 Hz), 5.248−5.182 (2H, m), 4.740−4.703 (1H, m), 4.016−3.965 (1H, m), 3.921 (1H, ddd, J = 11.1 Hz, 5.8 Hz, 3.4 Hz), 2.611 (1H, t, J = 7.6 Hz), 2.621 (2H, t, J = 7.6 Hz), 1.662−1.586 (2H, m), 1.395−1.302 (2H, m), 0.908 (3H, t, J = 7.3 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): 173.73, 170.41, 135.06, 128.65, 128.55, 128.17, 67.53, 63.68, 54.77, 36.21, 27.59, 22.30, 13.74. HRMS (ESI-TOF, [M + H]$^+$): calcd for $C_{15}H_{22}NO_4^+$: 280.1543; found, 280.1545. Anal. Calcd for $C_{15}H_{21}NO_4$: C, 64.50; H, 7.58; N, 5.01. Found: C, 64.84; H, 7.60; N, 5.05. mp 57.0−58.1 °C (colorless solids, recrystallized from *n*-hexane/CH_2Cl_2).

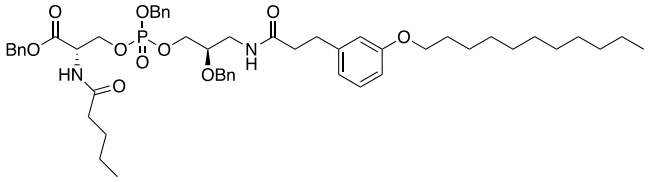
*Compound **45**.*



Compound **44** (282.8 mg, 1.012 mmol) was dissolved in CH_2Cl_2 and toluene. To remove traces of water, the solution was evaporated. Compound **3** (507.5 mg, 1.499 mmol) and anhydrous CH_2Cl_2 (4.0 mL) were added. A solution of 1*H*-tetrazole (73.5 mg, 1.05 mmol) in anhydrous THF (4.0 mL) was added at 0 °C. The mixture was stirred at room temperature under an Ar atmosphere for 3 h. A saturated aqueous solution of $NaHCO_3$ (15 mL) was added, and the whole was extracted three times with CH_2Cl_2 (15 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated to afford a colorless oil. The residue was column-chromatographed on an open column with silica gel (*n*-hexane/AcOEt/Et_3N = 40:1:1 to 40:5:1) to afford **45** as a colorless oil (393.0 mg, 0.7607 mmol, 75%).

^{1}H NMR (400 MHz, $CDCl_3$): 7.343−7.273 (10H, m), 6.561 (0.5H, J = 8.2 Hz) and 6.306 (0.5H, J = 8.0 Hz) (1H, 2d), 5.203−5.134 (2H, m), 4.814−4.557 (3H, m), 4.225−4.113 (1H, m), 3.924−3.815 (1H, m), 3.638−3.536 (2H, m), 2.183 (1H, J = 7.6 Hz) and 1.928 (1H, J = 7.6 Hz) (2H, 2t), 1.615−1.557 (1H, m), 1.527−1.448 (1H, m), 1.379−1.286 (1H, m), 1.267−1.125 (13H, m), 0.895 (1.5H, J = 7.3 Hz) and 0.843 (1.5H, J = 7.3 Hz) (3H, 2t). ^{13}C NMR (100 MHz, $CDCl_3$): 173.02, 172.88, 170.05, 169.97, 139.20, 139.12, 138.92, 138.84, 135.44, 135.28, 128.51, 128.50, 128.43, 128.30, 128.28, 128.24, 128.19, 128.05, 127.61, 127.37, 127.17, 126.95, 67.25, 67.12, 65.49, 65.31, 65.26, 65.08, 64.38, 64.22, 63.65, 63.50, 53.46, 53.40, 53.31, 43.19, 43.11, 43.07, 42.99, 36.23, 35.92, 27.60, 27.53, 24.65, 24.59, 24.57, 24.52, 24.45, 22.30, 22.21, 13.75. HRMS (ESI-TOF, [M + Na]$^+$): calcd for $C_{28}H_{41}N_2NaO_5P^+$, 539.2645; found, 539.2655.

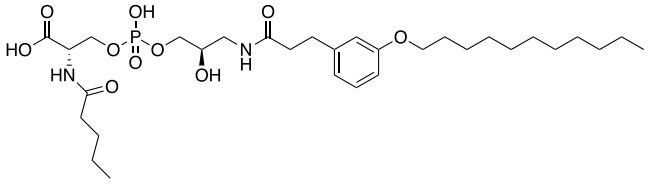
*Compound **46**.*



Compounds **45** (165.1 mg, 0.3196 mmol) and **2** (97.6 mg, 0.202 mmol) were dissolved in CH_2Cl_2 and toluene. To remove traces of water, the solution was evaporated. The mixture was dissolved in anhydrous CH_2Cl_2 (1.0 mL). A solution of 1*H*-tetrazole (27.8 mg, 0.397 mmol) in anhydrous THF (1.0 mL) was added at 0 °C. The mixture was stirred at room temperature under an Ar atmosphere for 6.5 h. TBHP in decane (5.0−6.0 M) (73 μL) was added, and the mixture was stirred at room temperature for 1.5 h. H_2O (10 mL) was added, and the whole was extracted three times with CH_2Cl_2 (10 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated to afford a colorless oil. The residue was column-chromatographed on a flash column with silica gel ($CHCl_3$/AcOEt = 10:1) to afford **46** as a colorless oil (128.5 mg, 0.1404 mmol, 70%).

^{1}H NMR (400 MHz, $CDCl_3$): (mixtures of diastereomers) 7.356−7.270 (15H, m), 7.175−7.128 (1H, m), 6.817−6.702 (4H, m), 5.917 (J = 6.1 Hz) and 5.850 (J = 5.9 Hz) (1H, 2t), 5.217−5.118 (2H, m), 5.031−4.945 (2H, m), 4.861−4.808 (1H, m), 4.585 (1H, d, J = 11.7 Hz), 4.466−4.386 (2H, m), 4.286−4.216 (1H, m), 4.026−3.838 (4H, m), 3.615−3.547 (1H, m), 3.404−3.294 (2H, m), 2.872 (2H, t, J = 7.8 Hz), 2.456−2.327 (2H, m), 2.227−2.160 (2H, m), 1.784−1.711 (2H, m), 1.619−1.535 (2H, m), 1.466−1.394 (2H, m), 1.355−1.265 (16H, m), 0.895−0.858 (6H, m). ^{13}C NMR (100 MHz, $CDCl_3$): (mixtures of diastereomers) 173.32, 173.30, 172.34, 172.33, 168.91, 168.89, 159.28, 142.28, 137.61, 137.57, 135.36, 135.29, 134.97, 129.39, 128.81, 128.78, 128.67, 128.60, 128.54, 128.52, 128.50, 128.27, 128.20, 128.06, 128.02, 127.97, 120.41, 114.68, 112.05, 75.54, 75.47, 75.43, 71.97, 69.84, 69.81, 69.79, 69.75, 67.86, 67.65, 67.54, 67.49, 67.44, 67.39, 66.64, 66.54, 66.47, 52.64, 52.58, 39.14, 38.10, 35.94, 31.87, 31.59, 29.58, 29.55, 29.40, 29.30, 27.47, 26.04, 22.65, 22.28, 14.08, 13.74. HRMS (ESI-TOF, [M + Na]$^+$): calcd for $C_{52}H_{71}N_2NaO_{10}P^+$, 937.4739; found, 937.4756.

*Compound **8c**.*



Compound **46** (112.2 mg, 0.1226 mmol) and Pd/C (22.9 mg) were dissolved in THF (5 mL). The mixture was stirred at room temperature under a H_2 atmosphere for 3 h. The mixture was filtered on Celite, and the solvent was evaporated. The residue was purified by reversed-phase medium-pressure liquid chromatography (CH_3CN/H_2O = 3:7 to 7:3, containing 0.1% HCOOH) to afford **8c** as a colorless powder (51.7 mg, 0.0802 mmol, 65%).

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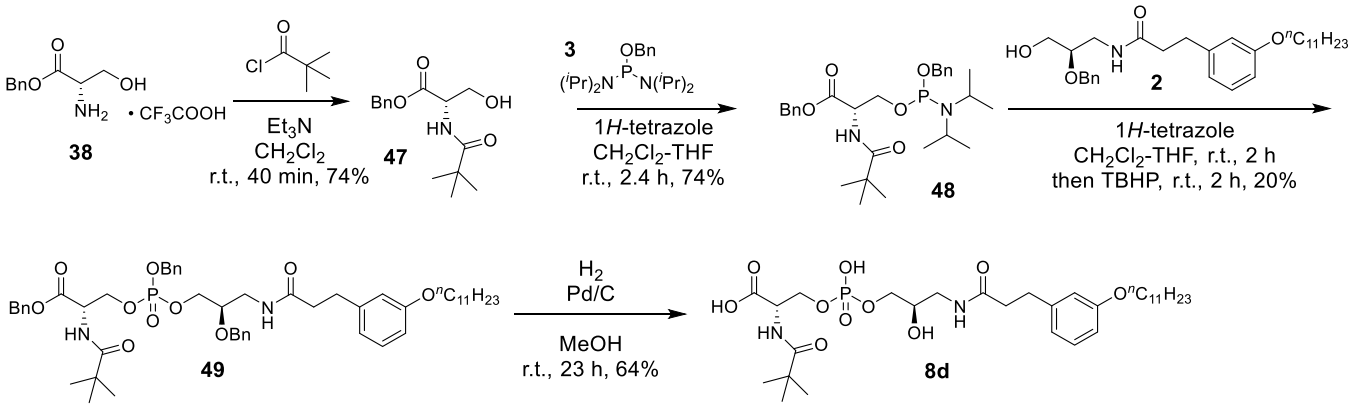
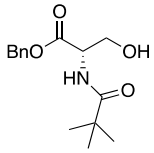


Figure 16. Synthesis of **8d**.

^{1}H NMR (400 MHz, DMSO-d_6): 8.267 (1H, d, J = 7.8 Hz), 7.874 (1H, t, J = 5.7 Hz), 7.149 (1H, dd, J = 8.1 Hz, 8.1 Hz), 6.753−6.700 (3H, m), 4.494−4.451 (1H, m), 4.123−4.017 (2H, m), 3.911 (2H, t, J = 6.5 Hz), 3.775−3.605 (3H, m), 3.188−3.127 (1H, m), 3.055−2.992 (1H, m), 2.758 (2H, t, J = 7.9 Hz), 2.378 (2H, t, J = 7.9 Hz), 2.147 (2H, t, J = 7.3 Hz), 1.683 (2H, quin, J = 6.9 Hz), 1.470 (2H, quin, J = 7.5 Hz), 1.405−1.357 (2H, m), 1.309−1.217 (16H, m), 0.869−0.832 (6H, m). HRMS (ESI-TOF, $[M - H]^-$): calcd for $C_{31}H_{52}N_2O_{10}P^-$, 643.3365; found, 643.3393. HPLC: 99% (CH_3CN/H_2O = 3:2 (0.1% HCO_2H)).

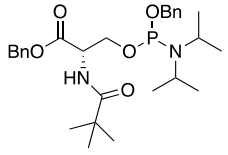
Synthesis of 8d (Figure 16). *Compound **47**.*



Compound **38** (620.8 mg, 2.007 mmol as TFA salt) and Et_3N (840 μL) were dissolved in anhydrous CH_2Cl_2 (8.0 mL). Pivaloyl chloride (0.27 mL) was added, and the mixture was stirred at room temperature under an Ar atmosphere for 40 min. MeOH (2 mL) was added, and the solvent was evaporated. The residue was column-chromatographed on an open column with silica gel (n-hexane/acetone = 3:1 to 2:1) to afford **47** as a yellow oil (415.1 mg, 1.486 mmol, 74%) (Figure 16).

^{1}H NMR (400 MHz, $CDCl_3$): 7.397−7.321 (5H, m), 6.618 (1H, d, J = 6.4 Hz), 5.255−5.179 (2H, m), 4.702−4.655 (1H, m), 3.985 (1H, dd, J = 11.1 Hz, 4.1 Hz), 3.927 (1H, dd, J = 11.1 Hz, 3.4 Hz), 2.364 (1H, br s), 1.226 (9H, s). ^{13}C NMR (100 MHz, $CDCl_3$): 179.23, 170.52, 135.07, 128.65, 128.54, 128.16, 67.52, 63.74, 54.90, 38.78, 27.40. HRMS (ESI-TOF, $[M + H]^+$): calcd for $C_{15}H_{22}NO_4^+$, 280.1543; found, 280.1535.

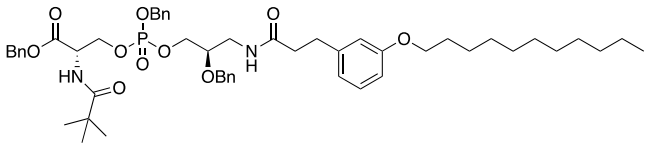
*Compound **48**.*



Compound **47** (278.0 mg, 0.9952 mmol) was dissolved in CH_2Cl_2 and toluene. To remove traces of water, the solution was evaporated. **3** (crude, 581.8 mg, 1.719 mmol) was added, and the mixture was dissolved in anhydrous CH_2Cl_2 (4.0 mL). A solution of 1H-tetrazole (74.4 mg, 1.06 mmol) in anhydrous THF (4.0 mL) was added at 0 °C. The mixture was stirred at room temperature under an Ar atmosphere for 2.4 h. A saturated aqueous solution of $NaHCO_3$ (10 mL) was added, and the whole was extracted three times with CH_2Cl_2 (10 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated to afford a colorless oil. The residue was column-chromatographed on an open column with silica gel (n-hexane/AcOEt/Et_3N = 40:1:1 to 40:4:1) to afford **48** as a colorless oil (380.8 mg, 0.7371 mmol, 74%).

^{1}H NMR (400 MHz, $CDCl_3$): 7.382−7.273 (10H, m), 6.650 (J = 8.0 Hz) and 6.534 (J = 7.8 Hz) (1H, 2d), 5.215−5.099 (2H, m), 4.777−4.547 (3H, m), 4.189−4.119 (1H, m), 3.939−3.836 (1H, m), 3.633−3.531 (2H, m), 1.191−1.124 (21H, m). ^{13}C NMR (100 MHz, $CDCl_3$): 178.34, 178.26, 170.16, 170.14, 139.21, 139.14, 139.08, 135.43, 135.35, 128.51, 128.35, 128.29, 128.27, 128.14, 128.09, 127.46, 127.37, 127.00, 126.95, 67.20, 67.17, 65.36, 65.33, 65.17, 63.82, 63.69, 63.54, 53.59, 53.53, 53.49, 53.42, 43.18, 43.16, 43.06, 43.03, 38.64, 38.57, 27.35, 27.28, 24.73, 24.65, 24.56, 24.50, 24.43. ^{31}P NMR (161 MHz, $CDCl_3$): 148.46, 148.41. HRMS (ESI-TOF, $[M + H]^+$): calcd for $C_{28}H_{42}N_2O_5P^+$, 517.2826; found, 517.2811.

*Compound **49**.*



Compounds **48** (366.0 mg, 0.7085 mmol) and **2** (202.2 mg, 0.4180 mmol) were dissolved in CH_2Cl_2 and toluene. To remove traces of water, the solution was evaporated. The mixture was dissolved in anhydrous CH_2Cl_2 (4.0 mL). A solution of 1H-tetrazole (60.1 mg, 0.858 mmol) in anhydrous THF (4.0 mL) was added. The mixture was stirred at room temperature under an Ar atmosphere for 2 h. TBHP in decane (5.0−6.0 M) (153 μL) was added, and the mixture was stirred at room temperature for 2 h. H_2O (10 mL) was added, and the whole was extracted three times with CH_2Cl_2 (10 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated to afford a colorless oil. The residue was column-chromatographed on a flash column with silica gel twice (first, $CHCl_3$/AcOEt = 3:2, second, $CHCl_3$/AcOEt = 3:1) to afford **49** as a colorless oil (pure fraction, 78.4 mg, 0.0857 mmol, 20%).

^{1}H NMR (400 MHz, $CDCl_3$): (mixtures of diastereomers) 7.351−7.276 (15H, m), 7.204−7.126 (1H, m), 7.011−6.936 (1H, m), 6.770−6.683 (3H, m), 5.976−5.864 (1H, m), 5.225−5.133 (2H, m), 5.016−4.922 (2H, m), 4.770−4.757 (1H, m), 4.703−4.667 (0.1H, m, rotamer of another peak?), 4.578 (1H, dd, J = 11.7 Hz, 2.2 Hz), 4.471−4.369 (2H, m), 4.321−4.237 (1H, m), 3.578 (1H, br s), 3.410−3.290 (2H, m), 2.987−2.949 and 2.887−2.849 (2H, 2m), 2.413−2.363 (2H, m), 1.744 (2H, quin, J = 6.7 Hz), 1.457−1.409 (2H, m), 1.346−1.262 (14H, m), 1.226 and 1.190 and 1.183 (9H, 3s), 0.879 (3H, t, J = 6.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): (mixtures of diastereomers)

https://doi.org/10.1021/acs.jmedchem.1c00347
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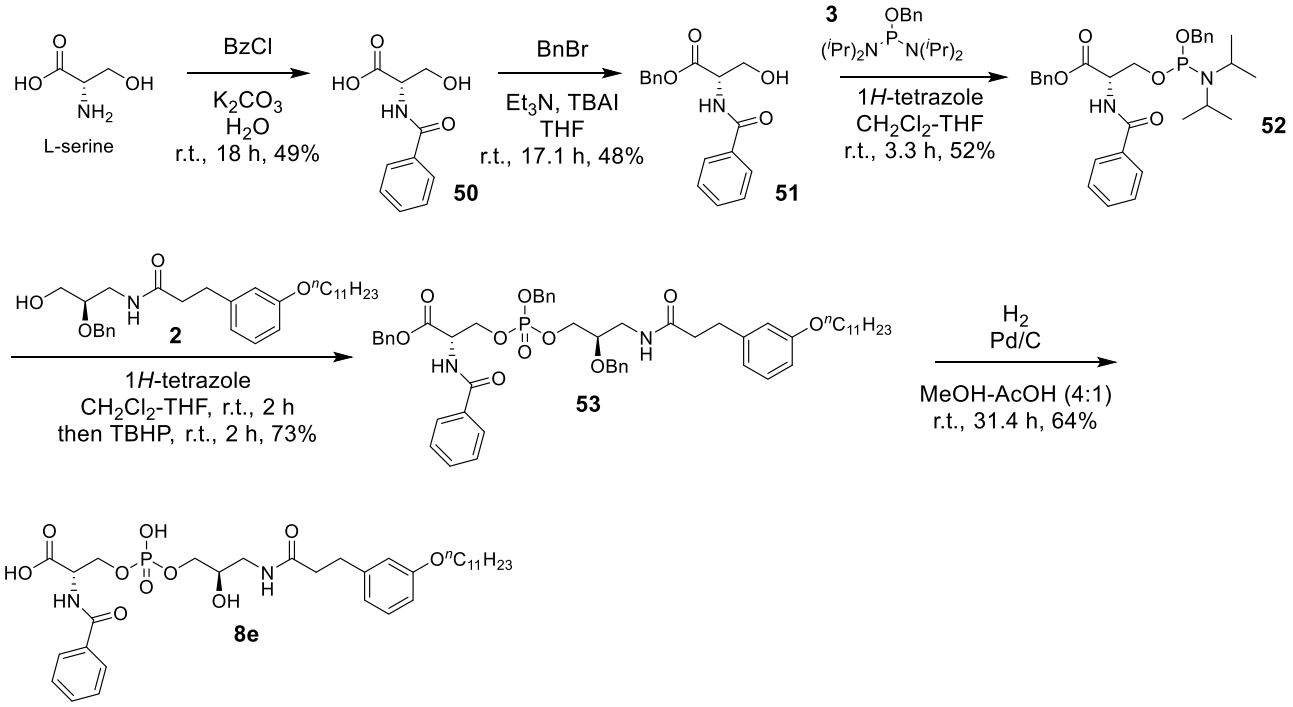
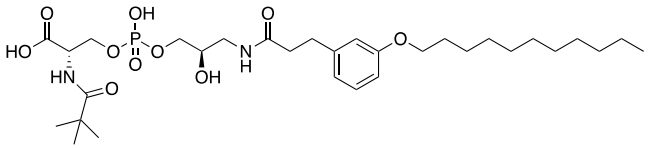


Figure 17. Synthesis of **8e**.

178.75, 178.72, 172.35, 168.98, 159.29, 142.29, 142.26, 137.66, 137.59, 135.30, 135.01, 129.41, 128.84, 128.79, 128.69, 128.67, 128.60, 128.55, 128.52, 128.50, 128.24, 128.19, 128.05, 128.02, 127.98, 120.42, 114.69, 112.07, 75.41, 71.98, 69.92, 69.86, 69.80, 67.87, 67.65, 67.62, 67.32, 66.78, 52.99, 39.12, 38.70, 38.09, 31.89, 31.61, 29.60, 29.57, 29.41, 29.31, 27.31, 26.06, 22.66, 14.10. HRMS (ESI-TOF, $[M + Na]^+$): calcd for $C_{52}H_{71}N_2NaO_{10}P^+$, 937.4739; found, 937.4729.

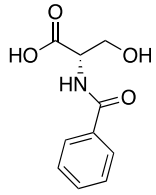
*Compound **8d**.*



Compound **49** (76.7 mg, 0.0838 mmol) and Pd/C (12.9 mg) were dissolved in MeOH (2 mL). The mixture was stirred at room temperature under a H_2 atmosphere for 23 h. The mixture was filtered on Celite, and the solvent was evaporated. The residue was purified by reversed-phase medium-pressure liquid chromatography (CH_3CN/H_2O = 3:7 to 7:3, containing 0.1% HCOOH) to afford **8d** as a colorless powder (34.7 mg, 0.0538 mmol, 64%).

^{1}H NMR (400 MHz, DMSO-d_6): 12.690 (1H, br s), 7.921−7.860 (2H, m), 7.149 (1H, dd, J = 8.1 Hz, 8.1 Hz), 6.753−6.700 (3H, m), 4.420−4.377 (1H, m), 4.164−4.126 (2H, m), 3.911 (2H, t, J = 6.5 Hz), 3.775−3.555 (3H, m), 3.184−3.123 (1H, m), 3.053−2.990 (1H, m), 2.757 (2H, t, J = 7.9 Hz), 2.375 (2H, t, J = 7.9 Hz), 1.684 (2H, quin, J = 7.0 Hz), 1.407−1.248 (16H, m), 1.150−1.112 (9H, m), 0.855 (3H, t, J = 6.9 Hz). HRMS (ESI-TOF, $[M - H]^-$): calcd for $C_{31}H_{52}N_2O_{10}P^-$, 643.3365; found, 643.3344. Anal. Calcd for $C_{31}H_{53}N_2O_{10}P{\cdot}0.5H_2O$: C, 56.95; H, 8.33; N, 4.29. Found: C, 56.97; H, 8.14; N, 4.07. HPLC: 97% (CH_3CN/H_2O = 3:2 (0.1% HCO_2H)).

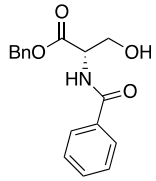
Synthesis of 8e (Figure 17). *Compound **50**.*



L-Serine (1051.8 mg, 10.008 mmol) and K_2CO_3 (4145.5 mg, 29.995 mmol) were dissolved in H_2O (34 mL). Benzyl chloride (1.7 mL) was added, and the whole was stirred at room temperature from 18 h. A 2 M aqueous solution of HCl was added to pH 3−4. The aqueous layer was extracted three times with AcOEt (40 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated to afford **50** as a colorless powder (1024.1 mg, 4.8953 mmol, 49%) (Figure 17).

^{1}H NMR (400 MHz, DMSO-d_6): 12.669 (1H, br s), 8.393 (1H, d, J = 7.7 Hz), 7.904−7.880 (2H, m), 7.575−7.532 (1H, m), 7.509−7.464 (2H, m), 4.981 (1H, br s), 4.501−4.456 (1H, m), 3.800 (2H, d, J = 5.1 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): 171.95, 166.38, 133.98, 131.42, 128.30, 127.39, 61.20, 55.64. HRMS (ESI-TOF, $[M - H]^-$): calcd for $C_{10}H_{10}NO_4^-$, 208.0615; found, 208.0615.

*Compound **51**.*



Compound **50** (1008.1 mg, 4.8188 mmol) was dissolved in anhydrous THF (40 mL). Triethylamine (2.0 mL), TBAI (531.6 mg, 1.443 mmol), and BnBr (630 μL) were added at 0 °C, and the mixture was stirred at room temperature under an Ar atmosphere for 17.1 h. H_2O (40 mL) was added, and the whole

https://doi.org/10.1021/acs.jmedchem.1c00347
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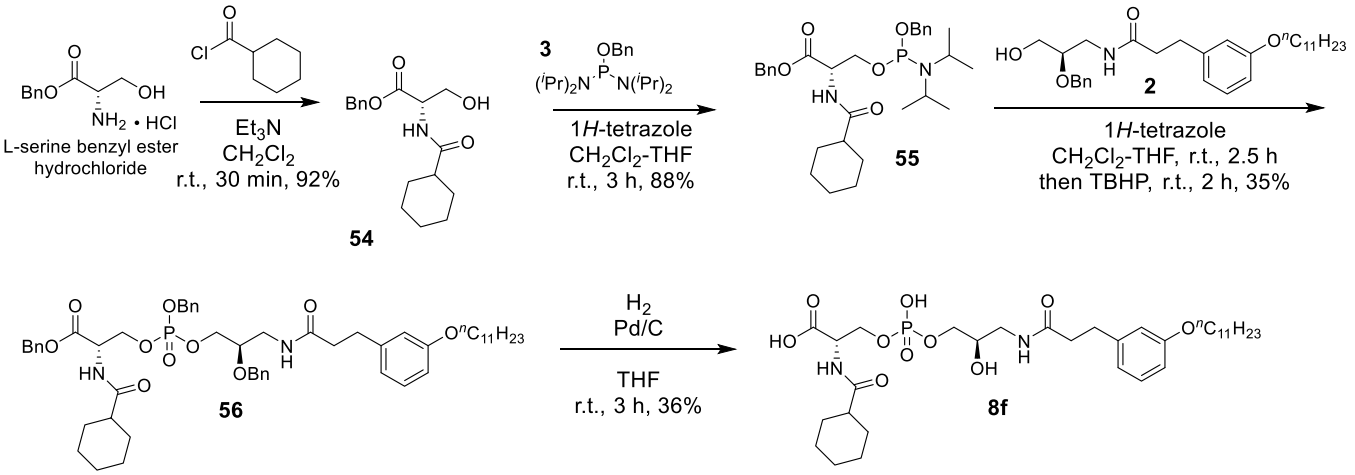
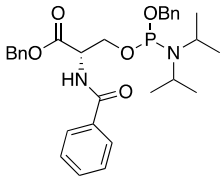


Figure 18. Synthesis of **8f**.

was extracted three times with AcOEt (40 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated to afford a yellow oil. The residue was column-chromatographed on an open column with silica gel (*n*-hexane/acetone = 1:1) to afford **51** as a colorless solid (698.1 mg, 2.332 mmol, 48%).

^{1}H NMR (400 MHz, $CDCl_3$): 7.822−7.801 (2H, m), 7.535−7.491 (1H, m), 7.445−7.406 (2H, m), 7.373−7.328 (5H, m), 7.139 (1H, d, *J* = 6.9 Hz), 5.278−5.213 (2H, m), 4.920−4.885 (1H, m), 4.108−4.025 (2H, m), 2.159 (1H, br s). ^{13}C NMR (100 MHz, $CDCl_3$): 170.44, 167.69, 135.05, 133.46, 131.99, 128.68, 128.63, 128.58, 128.19, 127.16, 67.66, 63.60, 55.32. HRMS (ESI-TOF, [M + H]$^+$): calcd for $C_{17}H_{18}NO_4^+$, 300.1230; found, 300.1239. mp 105.8−107.0 °C (colorless needles, recrystallized from *n*-hexane/CH_2Cl_2).

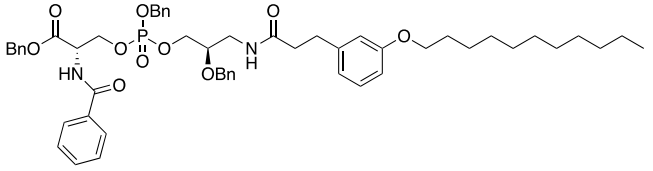
*Compound **52**.*



Compound **51** (150.9 mg, 0.5041 mmol) was dissolved in CH_2Cl_2 and toluene. To remove traces of water, the solution was evaporated. Compound **3** (crude, 292.4 mg, 0.8639 mmol) was added, and the mixture was dissolved in anhydrous CH_2Cl_2 (2.0 mL). A solution of 1*H*-tetrazole (37.2 mg, 0.531 mmol) in anhydrous THF (2.0 mL) was added at 0 °C. The mixture was stirred at room temperature under an Ar atmosphere for 3.3 h. A saturated aqueous solution of $NaHCO_3$ (10 mL) was added, and the whole was extracted three times with CH_2Cl_2 (10 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated to afford a colorless oil. The residue was column-chromatographed on an open column with silica gel (*n*-hexane/AcOEt/Et_3N = 40:2:1 to 40:6:1) to afford **52** as a colorless oil (140.4 mg, 0.2616 mmol, 52%).

^{1}H NMR (400 MHz, $CDCl_3$): 7.788−7.705 (2H, m), 7.523−7.376 (2H, m), 7.367−7.200 (11.5H, m), 7.039 (0.5H, d, *J* = 7.8 Hz), 5.220−5.216 (1H, m), 5.191 (1H, s), 5.105−4.948 (1H, m), 4.747−4.565 (2H, m), 4.303−4.204 (1H, m), 4.064−3.969 (1H, m), 3.644−3.529 (2H, m), 1.293−1.220 (1H, m, impurity), 1.150−1.108 (12H, m). HRMS (ESI-TOF, [M + H]$^+$): calcd for $C_{30}H_{38}N_2O_5P^+$, 537.2513; found, 537.2500.

*Compound **53**.*



Compounds **52** (118.7 mg, 0.2212 mmol) and **2** (63.8 mg, 0.132 mmol) were dissolved in CH_2Cl_2 and toluene. To remove traces of water, the solution was evaporated. The mixture was dissolved in anhydrous CH_2Cl_2 (1.0 mL). A solution of 1*H*-tetrazole (18.7 mg, 0.267 mmol) in anhydrous THF (1.0 mL) was added at 0 °C. The mixture was stirred at room temperature under an Ar atmosphere for 2 h. TBHP in decane (5.0−6.0 M) (47 μL) was added, and the mixture was stirred at room temperature for 2 h. H_2O (10 mL) was added, and the whole was extracted three times with CH_2Cl_2 (10 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated to afford a colorless oil. The residue was column-chromatographed on a flash column with silica gel ($CHCl_3$/AcOEt = 8:1) to afford **53** as a colorless oil (90.1 mg, 0.0964 mmol, 73%).

^{1}H NMR (400 MHz, $CDCl_3$): (mixtures of diastereomers) 7.877−7.839 (2H, m), 7.759 (*J* = 7.4 Hz) and 7.674 (*J* = 7.6 Hz) (1H, 2d), 7.495 (1H, dd, *J* = 7.4 Hz, 7.4 Hz), 7.420−7.382 (2H, m), 7.337−7.226 (15H, m), 7.164−7.114 (1H, m), 6.720−6.698 (3H, m), 5.881 (*J* = 5.7 Hz) and 5.787 (*J* = 5.7 Hz) (1H, 2t), 5.249−5.169 (2H, m), 5.020−4.921 (3H, m), 4.569−4.464 (2H, m), 4.431−4.370 (2H, m), 4.047−3.952 (1H, m), 3.908−3.820 (3H, m), 3.576−3.515 (1H, m), 3.344−3.232 (2H, m), 2.959−2.820 (2H, m), 2.490−2.303 (2H, m), 1.742 (2H, quin, *J* = 6.9 Hz), 1.439−1.262 (16H, m), 0.878 (3H, t, *J* = 6.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): (mixtures of diastereomers) 172.32, 168.85, 168.77, 167.18, 159.26, 142.26, 137.64, 137.53, 135.25, 135.20, 134.99, 134.97, 133.22, 133.18, 131.92, 129.39, 128.82, 128.74, 128.66, 128.63, 128.61, 128.58, 128.52, 128.51, 128.24, 128.19, 128.02, 127.98, 127.95, 127.33, 127.30, 120.41, 114.68, 114.66, 112.04, 75.50, 75.43, 75.34, 71.94, 70.01, 69.95, 69.90, 69.84, 67.85, 67.74, 67.72, 67.34, 66.85, 66.79, 66.71, 66.65, 53.54, 53.49, 53.43, 39.11, 39.07, 38.06, 38.02, 31.88, 31.57, 29.59, 29.56, 29.40, 29.30, 26.05, 22.66, 14.10. HRMS (ESI-TOF, [M + NH_4]$^+$): calcd for $C_{54}H_{71}N_3O_{10}P^+$, 952.4872; found, 952.4855.

https://doi.org/10.1021/acs.jmedchem.1c00347
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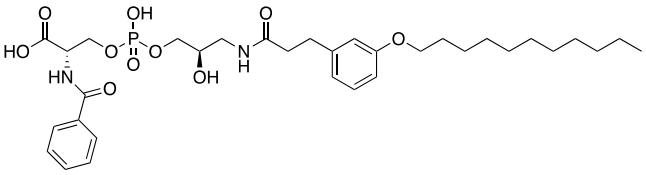
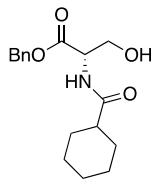
Compound ***8e***.



Compound **53** (81.0 mg, 0.0866 mmol) and Pd/C (8.0 mg) were dissolved in MeOH (4 mL) and AcOH (1 mL). The mixture was stirred at room temperature under a H_2 atmosphere for 31.4 h. The mixture was filtered on Celite, and the solvent was evaporated. The residue was purified by reversed-phase medium-pressure liquid chromatography (CH_3CN/H_2O = 3:7 to 7:3, containing 0.1% HCOOH) to afford **8e** as a colorless powder (36.7 mg, 0.0552 mmol, 64%).

^{1}H NMR (400 MHz, $CDCl_3$/TFA-*d*): 7.758 (2H, d, *J* = 7.4 Hz), 7.625 (1H, dd, *J* = 7.6 Hz, 7.6 Hz), 7.481 (2H, dd, *J* = 7.5 Hz, 7.5 Hz), 7.219 (1H, dd, *J* = 8.0 Hz, 8.0 Hz), 6.842−6.821 (1H, m), 6.763 (2H, br s), 5.132 (1H, br s), 4.508 (2H, br s), 4.142 (1H, br s), 4.041 (2H, t, *J* = 6.7 Hz), 3.932 (2H, br s), 3.637 (1H, br s), 3.187−3.155 (1H, m), 2.877 (2H, br s), 2.602 (2H, br s), 1.769 (2H, quin, *J* = 7.1 Hz), 1.450−1.264 (16H, m), 0.878 (3H, t, *J* = 6.8 Hz). HRMS (ESI-TOF, $[M - H]^-$): calcd for $C_{33}H_{48}N_2O_{10}P^-$, 663.3052; found, 663.3058. HPLC: 88% (CH_3CN/H_2O = 3:2 (0.1% HCO_2H)) (after purification by flash-column-chromatography ($CHCl_3$/acetone/AcOH = 2:1:1)).

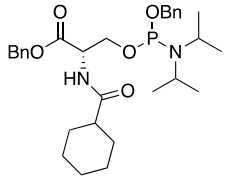
Synthesis of 8f (Figure 18). *Compound* ***54***.



L-Serine benzyl ester hydrochloride (349.5 mg, 1.509 mmol) was dissolved in anhydrous CH_2Cl_2 (6 mL). Et_3N (520 μL) and cyclohexanecarbonyl chloride (225 μL, 1.66 mmol) were added, and the mixture was stirred at room temperature under an Ar atmosphere for 30 min. MeOH (ca. 2 mL) was added, and the solvent was evaporated with silica gel. The residue was column-chromatographed on an open column with silica gel (*n*-hexane/acetone = 3:1 to 2:1) to afford **54** as a colorless solid (424.8 mg, 1.391 mmol, 92%) (Figure 18).

^{1}H NMR (400 MHz, $CDCl_3$): 7.396−7.320 (5H, m), 6.431 (1H, d, *J* = 6.9 Hz), 5.248−5.180 (2H, m), 4.723−4.687 (1H, m), 4.008−3.893 (2H, m), 2.614 (1H, t, *J* = 5.8 Hz), 2.213−2.137 (1H, m), 1.896−1.857 (2H, m), 1.801−1.769 (2H, m), 1.686−1.673 (1H, m), 1.496−1.380 (2H, m), 1.324−1.153 (3H, m). ^{13}C NMR (100 MHz, $CDCl_3$): 176.68, 170.46, 135.06, 128.64, 128.53, 128.17, 67.52, 63.78, 54.68, 45.19, 29.48, 25.64, 25.59, 25.57. HRMS (ESI-TOF, $[M + H]^+$): calcd for $C_{17}H_{24}NO_4^+$, 306.1700; found, 306.1680. Anal. Calcd for $C_{17}H_{23}NO_4$: C, 66.86; H, 7.59; N, 4.59. Found: C, 61.69; H, 7.47; N, 4.58. mp 106.4−108.5 °C (colorless powder, recrystallized from *n*-hexane/CH_2Cl_2).

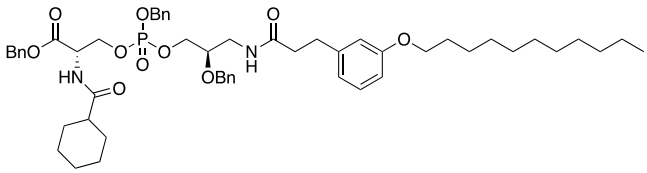
Compound ***55***.



Compound **54** (305.1 mg, 0.9991 mmol) was dissolved in CH_2Cl_2 and toluene. To remove traces of water, the solution was evaporated. Compound **3** (540.7 mg, 1.597 mmol) and anhydrous CH_2Cl_2 (4.0 mL) were added. A solution of 1*H*-tetrazole (72.6 mg, 1.04 mmol) in anhydrous THF (4.0 mL) was added at 0 °C. The mixture was stirred at room temperature under an Ar atmosphere for 3 h. A saturated aqueous solution of $NaHCO_3$ (15 mL) was added, and the whole was extracted three times with CH_2Cl_2 (15 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated to afford a colorless oil. The residue was column-chromatographed on an open column with silica gel (*n*-hexane/AcOEt/Et_3N = 40:1:1 to 40:5:1) to afford **55** as a colorless oil (478.1 mg, 0.8810 mmol, 88%).

^{1}H NMR (400 MHz, $CDCl_3$): 7.341−7.263 (10H, m), 6.525 (0.5H, *J* = 8.2 Hz) and 6.314 (0.5H, *J* = 8.1 Hz) (1H, 2d), 5.202−5.105 (2H, m), 4.797−4.554 (3H, m), 4.201−4.109 (1H, m), 3.925−3.801 (1H, m), 3.632−3.533 (2H, m), 2.121−2.041 and 1.916−1.580 (5H, m), 1.462−1.121 (17H, m). ^{13}C NMR (100 MHz, $CDCl_3$): 175.90, 175.79, 170.12, 170.06, 139.23, 139.16, 139.04, 135.43, 135.30, 128.49, 128.39, 128.27, 128.24, 128.19, 128.08, 127.51, 127.35, 126.92, 67.22, 67.13, 65.47, 65.29, 65.26, 65.08, 64.22, 64.06, 63.64, 63.49, 53.33, 53.27, 53.22, 53.15, 45.17, 44.90, 43.19, 43.12, 43.07, 43.00, 29.45, 29.42, 29.21, 25.70, 25.66, 25.61, 25.54, 25.48, 24.66, 24.60, 24.58, 24.52, 24.45. HRMS (ESI-TOF, $[M + H]^+$): calcd for $C_{30}H_{44}N_2O_5P^+$, 543.2982; found, 543.2967.

Compound ***56***.



Compounds **55** (185.0 mg, 0.3409 mmol) and **2** (97.2 mg, 0.201 mmol) were dissolved in CH_2Cl_2 and toluene. To remove traces of water, the solution was evaporated. The mixture was dissolved in anhydrous CH_2Cl_2 (2.0 mL). A solution of 1*H*-tetrazole (27.9 mg, 0.398 mmol) in anhydrous THF (2.0 mL) was added at 0 °C. The mixture was stirred at room temperature under an Ar atmosphere for 2.5 h. TBHP in decane (5.0−6.0 M) (73 μL) was added, and the mixture was stirred at room temperature for 2 h. H_2O (10 mL) was added, and the whole was extracted three times with CH_2Cl_2 (10 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated to afford a colorless oil. The residue was column-chromatographed on a flash column with silica gel twice ($CHCl_3$/AcOEt = 8:1) to afford **56** as a colorless oil (66.8 mg, 0.0710 mmol, 35%).

^{1}H NMR (400 MHz, $CDCl_3$): (mixtures of diastereomers) 7.350−7.266 (15H, m), 7.170−7.123 (1H, m), 6.761−6.679 (4H, m), 5.927 and 5.860 (1H, 2t, *J* = 5.9 Hz), 5.203−5.115 (2H, m), 5.022−4.935 (2H, m), 4.838−4.788 (1H, m), 4.581 (1H, d, *J* = 11.6 Hz), 4.465−4.375 (2H, m), 4.275−4.216 (1H, m), 4.023−3.836 (4H, m), 3.595−3.545 (1H, m), 3.388−3.300 (2H, m), 2.869 (2H, t, *J* = 7.8 Hz), 2.446−2.341 (2H, m), 2.174−2.064 (1H, m), 1.832−1.619 (7H, m), 1.425−1.179 (21H, m), 0.876 (3H, t, *J* = 6.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): (mixtures of diastereomers) 176.20, 176.18, 172.28, 168.97, 168.95, 159.27, 142.29, 137.63, 137.59, 135.36, 135.29, 134.97, 129.38, 128.79, 128.75, 128.66, 128.58, 128.53, 128.51, 128.48, 128.26, 128.20, 128.03, 128.00, 127.96, 127.93, 120.40, 114.67, 112.04, 75.53, 75.46, 75.42, 71.96, 69.82, 69.78, 69.73, 67.85, 67.62, 67.52, 67.47, 66.68, 66.61, 66.54, 52.55, 52.48, 44.89, 39.11, 39.08, 38.11, 31.87, 31.59, 29.57, 29.55, 29.39, 29.29, 26.04, 25.62, 25.56, 25.52, 22.64, 14.08. HRMS (ESI-TOF, $[M + Na]^+$): calcd for $C_{54}H_{73}N_2NaO_{10}P^+$, 963.4895; found, 963.4880.

https://doi.org/10.1021/acs.jmedchem.1c00347
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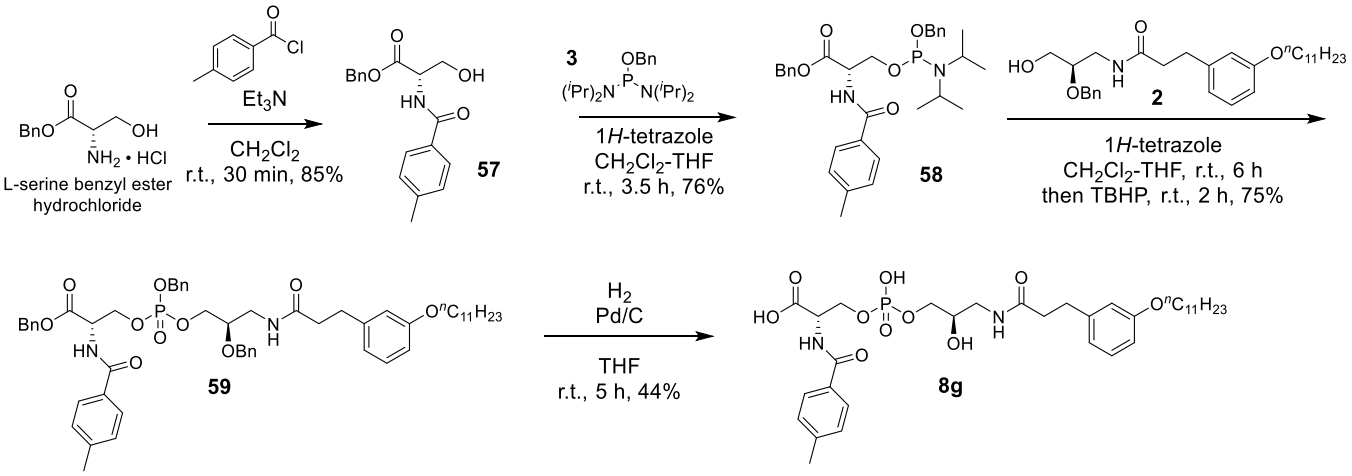
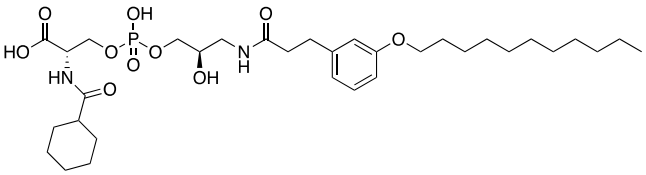


Figure 19. Synthesis of **8g**.

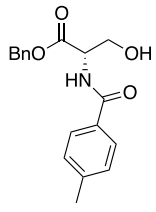
*Compound **8f**.*



Compound **56** (58.5 mg, 0.0622 mmol) and Pd/C (20.9 mg) were dissolved in THF (5 mL). The mixture was stirred at room temperature under a H_2 atmosphere for 3 h. The mixture was filtered on Celite, and the solvent was evaporated. The residue was purified by reversed-phase medium-pressure liquid chromatography (CH_3CN/H_2O = 3:7 to 7:3, containing 0.1% HCOOH) to afford **8f** as a colorless powder (15.1 mg, 0.0225 mmol, 36%).

^{1}H NMR (400 MHz, DMSO-d_6): 8.213 (1H, d, J = 7.8 Hz), 7.881 (1H, t, J = 5.6 Hz), 7.147 (1H, dd, J = 8.1 Hz, 8.1 Hz), 6.752−6.698 (3H, m), 4.446−4.403 (1H, m), 4.106−4.002 (2H, m), 3.909 (2H, t, J = 6.5 Hz), 3.758−3.590 (3H, m), 3.179−3.117 (1H, m), 3.056−2.992 (1H, m), 2.757 (2H, t, J = 7.9 Hz), 2.375 (2H, t, J = 7.9 Hz), 2.229−2.173 (1H, m), 1.699−1.581 (7H, m), 1.406−1.108 (21H, m), 0.853 (3H, t, J = 6.8 Hz). HRMS (ESI-TOF, [M − H]$^-$): calcd for $C_{33}H_{54}N_2O_{10}P^-$, 669.3522; found, 669.3506. HPLC: >99% (CH_3CN/H_2O = 3:2 (0.1% HCO_2H)).

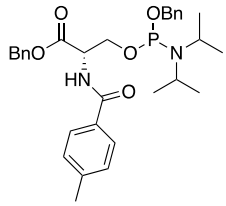
Synthesis of 8g (Figure 19). *Compound **57**.*



L-Serine benzyl ester hydrochloride (693.5 mg, 2.993 mmol) was dissolved in anhydrous CH_2Cl_2 (10 mL). Et_3N (1040 μL) and *p*-toluoyl chloride (440 μL, 3.33 mmol) were added, and the mixture was stirred at room temperature under an Ar atmosphere for 30 min. MeOH (ca. 2 mL) was added, and the solvent was evaporated with silica gel. The residue was column-chromatographed on an open column with silica gel (*n*-hexane/acetone = 3:1 to 2:1) to afford **57** as a colorless solid (794.4 mg, 2.535 mmol, 85%) (Figure 19).

^{1}H NMR (400 MHz, $CDCl_3$): 7.722 (2H, d, J = 8.2 Hz), 7.384−7.318 (5H, m), 7.242 (2H, d, J = 7.9 Hz), 7.072 (1H, d, J = 6.7 Hz), 5.294−5.227 (2H, m), 4.927−4.891 (1H, m), 4.076 (2H, br s), 2.518 (1H, br s), 2.402 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$): 170.50, 167.65, 142.51, 135.08, 130.60, 129.28, 128.67, 128.55, 128.17, 127.17, 67.62, 63.73, 55.33, 21.47. HRMS (ESI-TOF, [M + H]$^+$): calcd for $C_{18}H_{20}NO_4^+$, 314.1387; found, 314.1396. mp 122.0−123.4 °C (colorless needles, recrystallized from *n*-hexane/CH_2Cl_2).

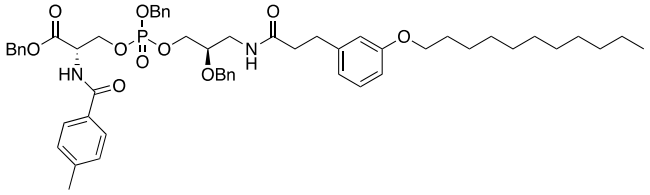
*Compound **58**.*



Compound **57** (312.5 mg, 0.9973 mmol) was dissolved in CH_2Cl_2 and toluene. To remove traces of water, the solution was evaporated. Compound **3** (540.7 mg, 1.597 mmol) and anhydrous CH_2Cl_2 (4.0 mL) were added. A solution of 1*H*-tetrazole (73.9 mg, 1.05 mmol) in anhydrous THF (4.0 mL) was added at 0 °C. The mixture was stirred at room temperature under an Ar atmosphere for 3.5 h. A saturated aqueous solution of $NaHCO_3$ (15 mL) was added, and the whole was extracted three times with CH_2Cl_2 (15 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated to afford a colorless oil. The residue was column-chromatographed on an open column with silica gel (*n*-hexane/AcOEt/Et_3N = 40:1:1, 40:4:1 to 40:5:1) to afford **58** as a colorless oil (417.4 mg, 0.7580 mmol, 76%).

^{1}H NMR (400 MHz, $CDCl_3$): 7.677 and 7.618 (2H, 2d, J = 8.2 Hz), 7.356−7.197 (11.5 H, m), 7.122 (1H, d, J = 8.0 Hz), 7.010 (0.5H, d, J = 7.8 Hz), 5.211 and 5.184 (2H, 2s), 5.010−4.942 (1H, m), 4.740−4.561 (2H, m), 4.288−4.196 (1H, m), 4.052−3.954 (1H, m), 3.640−3.527 (2H, m), 2.390 and 2.364 (3H, 2s), 1.148−1.106 (12H, m). HRMS (ESI-TOF, [M + H]$^+$): calcd for $C_{31}H_{40}N_2O_5P^+$, 551.2669; found, 551.2664.

*Compound **59**.*



Compounds **58** (186.2 mg, 0.3382 mmol) and **2** (96.9 mg, 0.200 mmol) were dissolved in CH_2Cl_2 and toluene. To remove traces

https://doi.org/10.1021/acs.jmedchem.1c00347
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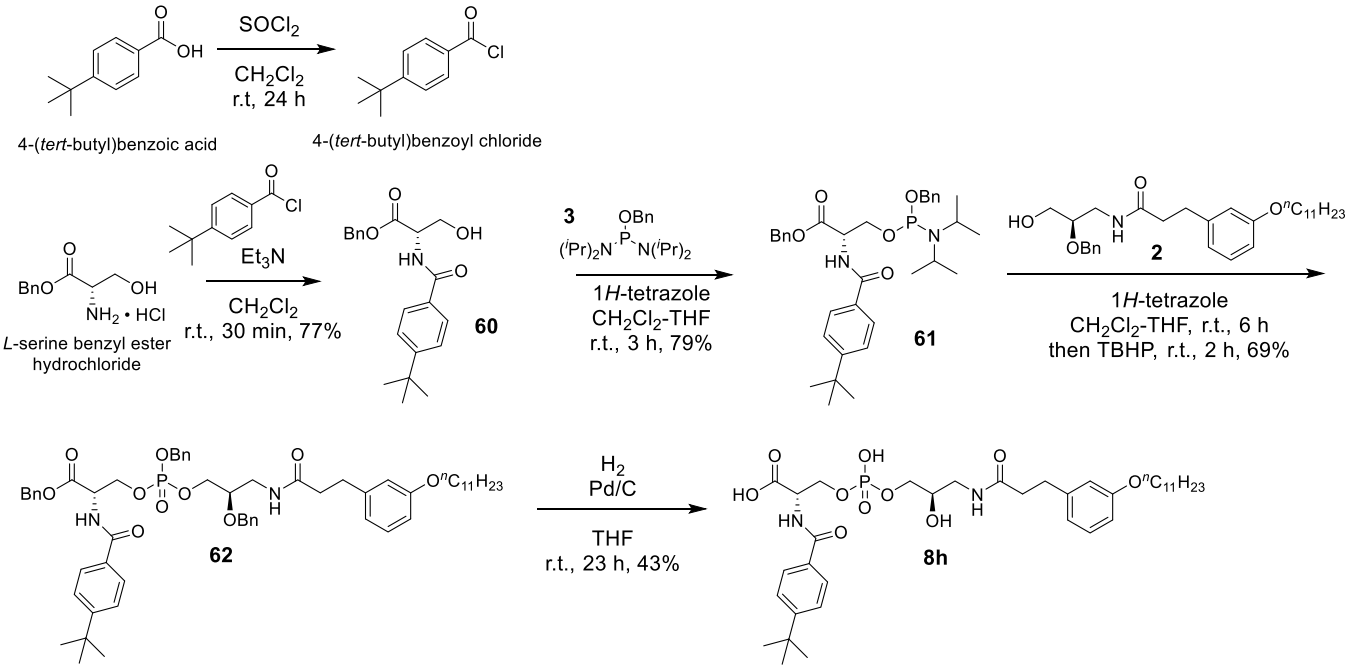
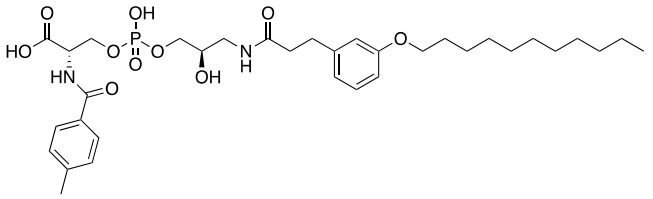


Figure 20. Synthesis of **8h**.

of water, the solution was evaporated. The mixture was dissolved in anhydrous CH_2Cl_2 (1.0 mL). A solution of 1*H*-tetrazole (27.5 mg, 0.393 mmol) in anhydrous THF (1.0 mL) was added at 0 °C. The mixture was stirred at room temperature under an Ar atmosphere for 6 h. TBHP in decane (5.0−6.0 M) (73 μL) was added, and the mixture was stirred at room temperature for 2 h. H_2O (10 mL) was added, and the whole was extracted three times with CH_2Cl_2 (10 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated to afford a colorless oil. The residue was column-chromatographed on a flash column with silica gel twice ($CHCl_3$/AcOEt = 10:1) to afford **59** as a colorless oil (142.5 mg, 0.1501 mmol, 75%).

^{1}H NMR (400 MHz, $CDCl_3$): (mixtures of diastereomers) 7.766−7.729 (2H, m), 7.661 (*J* = 7.4 Hz) and 7.563 (*J* = 7.6 Hz) (1H, 2d), 7.360−7.226 (15H, m), 7.193 (2H, d, *J* = 8.0 Hz), 7.163−7.114 (1H, m), 6.720−6.693 (3H, m), 5.882 (*J* = 5.9 Hz) and 5.781 (*J* = 5.9 Hz) (1H, 2t), 5.236−5.164 (2H, m), 5.017−4.918 (3H, m), 4.566−4.456 (2H, m), 4.431−4.363 (2H, m), 4.041−3.950 (1H, m), 3.908−3.818 (3H, m), 3.589−3.450 (1H, m), 3.341−3.245 (2H, m), 2.838 (2H, t, *J* = 7.6 Hz), 2.370−2.314 (5H, m), 1.740 (2H, quin, *J* = 6.8 Hz), 1.459−1.388 (2H, m), 1.342−1.261 (14H, m), 0.877 (3H, t, *J* = 6.9 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): (mixtures of diastereomers) 172.31, 172.26, 168.96, 168.88, 167.14, 159.28, 142.43, 142.33, 142.32, 137.69, 137.58, 135.31, 135.25, 135.03, 135.01, 130.42, 130.39, 129.39, 129.26, 128.81, 128.74, 128.67, 128.62, 128.53, 128.50, 128.25, 128.19, 128.03, 128.00, 127.97, 127.33, 127.31, 120.42, 114.70, 114.68, 112.05, 75.52, 75.45, 75.37, 71.94, 69.99, 69.93, 69.88, 69.82, 67.87, 67.73, 67.71, 67.42, 66.85, 66.79, 66.64, 53.50, 53.43, 53.37, 39.12, 39.06, 38.08, 38.05, 31.89, 31.58, 29.60, 29.57, 29.42, 29.32, 26.06, 22.67, 21.47, 14.10. HRMS (ESI-TOF, $[M + NH_4]^+$): calcd for $C_{55}H_{73}N_3O_{10}P^+$, 966.5028; found, 966.5032.

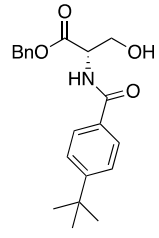
*Compound **8g**.*



Compound **59** (106.6 mg, 0.1123 mmol) and Pd/C (24.3 mg) were dissolved in THF (5 mL). The mixture was stirred at room temperature under a H_2 atmosphere for 5 h. The mixture was filtered on Celite, and the solvent was evaporated. The residue was purified by reversed-phase medium-pressure liquid chromatography (CH_3CN/H_2O = 3:7 to 7:3, containing 0.1% HCOOH). Because the HRMS spectrum showed partial decomposition of the product at the phosphodiester part, the product was further purified by a flash column with silica gel ($CHCl_3$/acetone/AcOH = 4:1:1 to 2:1:1) to afford **8g** as a colorless powder (33.2 mg, 0.0489 mmol, 44%).

^{1}H NMR (400 MHz, $CDCl_3$/TFA-*d*): 7.650 (2H, d, *J* = 7.8 Hz), 7.286−7.198 (3H, m), 6.839−6.819 (1H, m), 6.766 (2H, br s), 5.112 (1H, br s), 4.519 (2H, br s), 4.139 (1H, br s), 4.040 (2H, t, *J* = 6.8 Hz), 3.937 (2H, br s), 3.591 (1H, br s), 3.223 (1H, d, *J* = 13.7 Hz), 2.881 (2H, t, *J* = 6.9 Hz), 2.610 (2H, t, *J* = 6.9 Hz), 2.416 (3H, s), 1.767 (2H, quin, *J* = 7.1 Hz), 1.471−1.398 (2H, m), 1.300−1.266 (14H, m), 0.878 (3H, t, *J* = 6.8 Hz). HRMS (ESI-TOF, $[M - H]^-$): calcd for $C_{34}H_{50}N_2O_{10}P^-$, 677.3209; found, 677.3229. HPLC: 94% (CH_3CN/H_2O = 3:2 (0.1% HCO_2H)).

Synthesis of 8h (Figure 20). *Compound **60**.*



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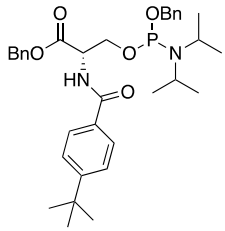
4-*tert*-Butylbenzoic acid (589.0 mg, 3.305 mmol) was dissolved in anhydrous CH_2Cl_2 (7.0 mL). $SOCl_2$ (480 μL, 6.62 mmol) was added to the solution, and the whole was stirred at room temperature under an Ar atmosphere for 24 h. The solvent was evaporated to afford crude 4-(*tert*-butyl)benzoyl chloride as a colorless oil and powder (648.6 mg), which was used without further purification (Figure 20).

L-Serine benzyl ester hydrochloride (693.3 mg, 2.993 mmol) was dissolved in anhydrous CH_2Cl_2 (5 mL). Et_3N (1040 μL), crude 4-(*tert*-butyl)benzoyl chloride (648.6 mg), and CH_2Cl_2 (5 mL) were added, and the mixture was stirred at room temperature under an Ar atmosphere for 30 min. MeOH (ca. 2 mL) was added, and the solvent was evaporated with silica gel. The residue was column-chromatographed on an open column with silica gel twice (first, *n*-hexane/acetone = 2:1, second, *n*-hexane/acetone = 3:1 to 0:1) to afford **60** as a colorless solid (821.7 mg, 2.312 mmol, 77%).

^{1}H NMR (400 MHz, $CDCl_3$): 7.764 (2H, d, J = 8.6 Hz), 7.453 (2H, d, J = 8.6 Hz), 7.398−7.317 (5H, m), 7.101 (1H, d, J = 6.9 Hz), 5.290−5.223 (2H, m), 4.931−4.895 (1H, m), 4.106−4.035 (2H, m), 2.601 (1H, br s), 1.333 (9H, s). ^{13}C NMR (100 MHz, $CDCl_3$): 170.48, 167.62, 155.58, 135.08, 130.56, 128.67, 128.55, 128.16, 127.02, 125.56, 67.62, 63.77, 55.33, 34.96, 31.12. HRMS (ESI-TOF, $[M + Na]^+$): calcd for $C_{21}H_{25}NNaO_4^+$, 378.1676; found, 378.1674. Anal. Calcd for $C_{21}H_{25}NO_4$: C, 70.96; H, 7.09; N, 3.94. Found: C, 71.28; H, 7.20; N, 4.03. mp 124.5−125.1 °C (colorless plates, recrystallized from *n*-hexane/CH_2Cl_2).

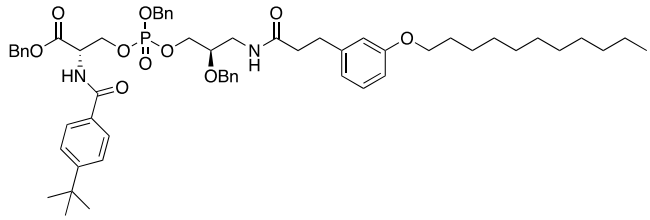
Compound ***61***.



Compound **60** (356.3 mg, 1.002 mmol) was dissolved in CH_2Cl_2 and toluene. To remove traces of water, the solution was evaporated. **3** (541.4 mg, 1.600 mmol) and anhydrous CH_2Cl_2 (4.0 mL) were added. A solution of 1*H*-tetrazole (72.9 mg, 1.04 mmol) in anhydrous THF (4.0 mL) was added at 0 °C. The mixture was stirred at room temperature under an Ar atmosphere for 3 h. A saturated aqueous solution of $NaHCO_3$ (15 mL) was added, and the whole was extracted three times with CH_2Cl_2 (15 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated to afford a colorless oil. The residue was column-chromatographed on an open column with silica gel (*n*-hexane/AcOEt/Et_3N = 40:1:1 to 40:4:1) to afford **61** as a colorless oil (466.9 mg, 0.7877 mmol, 79%).

^{1}H NMR (400 MHz, $CDCl_3$): 7.714 and 7.651 (2H, 2d, J = 8.5 Hz), 7.417 (1H, d, J = 8.6 Hz), 7.359−7.211 (11.5H, m), 7.015 (0.5H, d, J = 7.9 Hz), 5.212−5.210 (1H, m), 5.183 (1H, s), 5.014−4.951 (1H, m), 4.748−4.586 (2H, m), 4.299−4.197 (1H, m), 4.058−3.953 (1H, m), 3.648−3.533 (2H, m), 1.332 and 1.310 (9H, 2s), 1.154−1.117 (12H, m). ^{13}C NMR (100 MHz, $CDCl_3$): 170.08, 169.99, 167.00, 166.98, 155.17, 154.97, 139.12, 138.98, 138.91, 135.45, 135.31, 131.00, 130.96, 128.52, 128.30, 128.29, 128.26, 128.23, 128.17, 128.03, 127.38, 127.32, 126.95, 126.93, 125.42, 125.35, 67.29, 67.19, 65.52, 65.38, 65.34, 65.20, 64.32, 64.16, 63.65, 63.50, 53.99, 53.93, 53.86, 43.24, 43.14, 43.12, 43.02, 34.90, 34.85, 31.12, 24.66, 24.62, 24.58, 24.54, 24.51, 24.47, 24.44, 24.40. HRMS (ESI-TOF, $[M + Na]^+$): calcd for $C_{34}H_{45}N_2NaO_5P^+$, 615.2958; found, 615.2987.

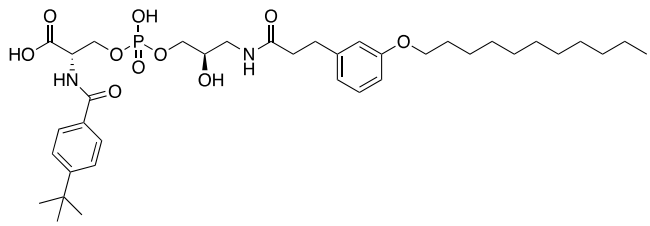
Compound ***62***.



Compounds **61** (201.3 mg, 0.3396 mmol) and **2** (97.9 mg, 0.202 mmol) were dissolved in CH_2Cl_2 and toluene. To remove traces of water, the solution was evaporated. The mixture was dissolved in anhydrous CH_2Cl_2 (1.0 mL). A solution of 1*H*-tetrazole (27.5 mg, 0.393 mmol) in anhydrous THF (1.0 mL) was added at 0 °C. The mixture was stirred at room temperature under an Ar atmosphere for 6 h. TBHP in decane (5.0−6.0 M) (73 μL) was added, and the mixture was stirred at room temperature for 2 h. H_2O (10 mL) was added, and the whole was extracted three times with CH_2Cl_2 (10 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated to afford a colorless oil. The residue was column-chromatographed on a flash column with silica gel ($CHCl_3$/AcOEt = 10:1) to afford **62** as a colorless oil (139.1 mg, 0.1403 mmol, 69%).

^{1}H NMR (400 MHz, $CDCl_3$): (mixtures of diastereomers) 7.819−7.780 (2H, m), 7.694 (0.5H, J = 7.4 Hz) and 7.601 (0.5H, J = 7.6 Hz) (1H, 2d), 7.432−7.405 (2H, m), 7.335−7.225 (15H, m), 7.160−7.109 (1H, m), 6.720−6.686 (3H, m), 5.896 and 5.811 (1H, 2t, J = 5.9 Hz), 5.237−5.165 (2H, m), 5.022−4.919 (3H, m), 4.574−4.458 (2H, m), 4.435−4.366 (2H, m), 4.043−3.960 (1H, m), 3.905−3.825 (3H, m), 3.597−3.512 (1H, m), 3.310−3.281 (2H, m), 2.863−2.818 (2H, m), 2.375−2.328 (2H, m), 1.774−1.721 (2H, m), 1.467−1.387 (2H, m), 1.342−1.261 (24H, m), 0.878 (3H, t, J = 6.9 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): (mixtures of diastereomers) 172.31, 172.27, 168.95, 168.87, 167.12, 167.10, 159.28, 155.47, 155.45, 142.32, 142.30, 137.69, 137.57, 135.30, 135.25, 135.04, 135.01, 130.37, 130.33, 129.39, 128.79, 128.71, 128.66, 128.62, 128.52, 128.49, 128.24, 128.18, 128.02, 127.97, 127.16, 125.53, 120.42, 114.69, 114.67, 112.05, 75.49, 75.43, 75.38, 71.94, 69.98, 69.92, 69.88, 69.82, 67.86, 67.71, 67.69, 67.46, 67.41, 66.86, 66.80, 66.73, 66.67, 53.50, 53.44, 53.38, 39.11, 39.08, 38.10, 38.05, 34.93, 31.88, 31.58, 31.11, 29.59, 29.57, 29.41, 29.31, 26.05, 22.66, 14.09. HRMS (ESI-TOF, $[M + NH_4]^+$): calcd for $C_{58}H_{79}N_3O_{10}P^+$, 1008.5498; found, 1008.5515.

Compound ***8h***.



Compound **62** (113.1 mg, 0.1141 mmol) and Pd/C (26.5 mg) were dissolved in THF (5 mL). The mixture was stirred at room temperature under a H_2 atmosphere for 23 h. The mixture was filtered on Celite, and the solvent was evaporated. The residue was purified by reversed-phase medium-pressure liquid chromatography (CH_3CN/H_2O = 35:65 to 7:3, containing 0.1% HCOOH). Because the HRMS spectrum showed partial decomposition of the product at the phosphodiester part, the product was further purified by a flash column with silica gel ($CHCl_3$/acetone/AcOH = 2:1:1) to afford **5l** as a colorless powder (35.2 mg, 0.0488 mmol, 43%).

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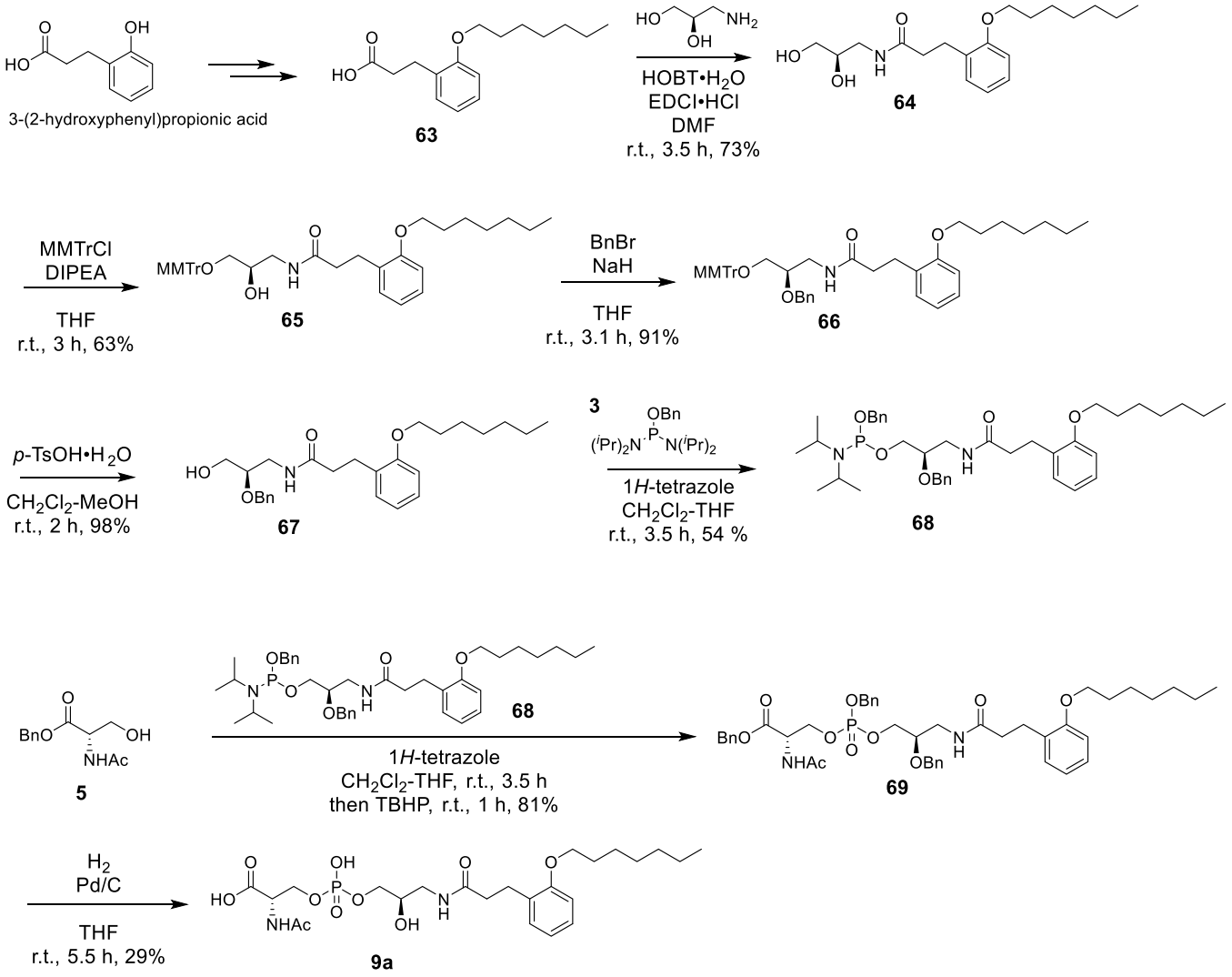
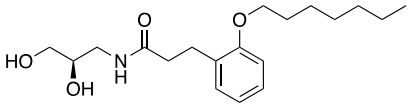


Figure 21. Synthesis of **9a**.

^{1}H NMR (400 MHz, $CDCl_3$/TFA-*d*): 7.691 (2H, d, *J* = 7.8 Hz), 7.499 (2H, d, *J* = 8.2 Hz), 7.216 (1H, dd, *J* = 8.0 Hz, 8.0 Hz), 6.839–6.767 (3H, m), 5.124 (1H, br s), 4.522 (2H, br s), 4.153 (1H, br s), 4.042 (2H, t, *J* = 6.7 Hz), 3.947 (2H, br s), 3.612 (1H, br s), 3.439 (br s) and 3.215 (d, *J* = 13.4 Hz), 2.880 (2H, t, *J* = 7.0 Hz), 2.628–2.611 (2H, m), 1.766 (2H, quin, *J* = 7.1 Hz), 1.424–1.263 (25H, m), 0.876 (3H, t, *J* = 6.8 Hz). HRMS (ESI-TOF, $[M - H]^-$): calcd for $C_{37}H_{56}N_2O_{10}P^-$, 719.3678; found, 719.3656. HPLC: 86% (CH_3CN/H_2O = 3:2 (0.1% HCO_2H)).

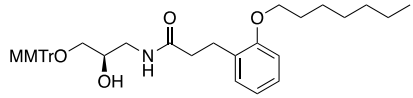
Synthesis of 9a (Figure 21). *Compound **64**.*



Compounds **63** (1270.5 mg, 4.8059 mmol) and 1-hydroxybenzotriazole monohydrate (777.6 mg, 5.754 mmol) were dissolved in anhydrous DMF (5 mL). (*R*)-3-Amino-1,2-propanediol (481.0 mg, 5.279 mmol) was added to the mixture with anhydrous DMF (10 mL). Then, EDCI·HCl (1102.5 mg, 5.7511 mmol) was added at 0 °C, and the whole was stirred at room temperature under an Ar atmosphere for 3.5 h. A saturated aqueous solution of $NaHCO_3$ (30 mL) was added. The mixture was filtered to obtain the precipitate, **64**, as a colorless solid (664.1 mg, 1.968 mmol, 73%) (Figure 21).

^{1}H NMR (400 MHz, DMSO-d_6): 7.779 (1H, t, *J* = 5.7 Hz), 7.158–7.095 (2H, m), 6.906 (1H, d, *J* = 7.5 Hz), 6.820 (1H, ddd, *J* = 7.4 Hz, 7.4 Hz, 1.0 Hz), 4.732 (1H, br s), 4.517 (1H, br s), 3.950 (2H, t, *J* = 6.4 Hz), 3.479–3.423 (1H, m), 3.257–3.240 (2H, m), 3.207–3.146 (1H, m), 3.002–2.938 (1H, m), 2.760 (2H, t, *J* = 7.8 Hz), 2.347 (2H, t, *J* = 7.9 Hz), 1.730 (2H, quin, *J* = 6.9 Hz), 1.469–1.397 (2H, m), 1.369–1.257 (6H, m), 0.868 (3H, t, *J* = 6.9 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): 172.09, 156.38, 129.28, 127.18, 120.03, 111.34, 70.53, 67.34, 63.59, 42.08, 35.16, 31.23, 28.77, 28.43, 25.67, 25.54, 22.04, 13.95. HRMS (ESI-TOF, $[M + H]^+$): calcd for $C_{19}H_{32}NO_4^+$, 338.2326; found, 338.2325.

*Compound **65**.*



Compound **64** (1101.6 mg, 3.2644 mmol) was dissolved in anhydrous THF (10 mL). DIPEA (1.7 mL) and MMTrCl (1306.4 mg, 4.2305 mmol) were added to the solution, and the whole was stirred at room temperature under an Ar atmosphere for 3 h. A saturated aqueous solution of $NaHCO_3$ (20 mL) was added to the solution, and the whole was extracted three times with AcOEt (20 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated to afford a yellow oil. The residue was column-

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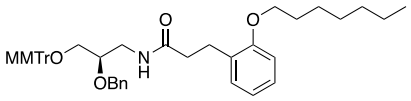
chromatographed on an open column with silica gel (*n*-hexane/AcOEt = 3:1 to 3:2) to afford **65** as a pale yellow sticky oil (1293.8 mg, 2.0446 mmol, 63%).

^{1}H NMR (400 MHz, $CDCl_3$): 7.413−7.383 (4H, m), 7.305−7.258 (6H, m), 7.238−7.196 (2H, m), 7.159 (1H, ddd, J = 7.8 Hz, 7.8 Hz, 1.7 Hz), 7.101 (1H, dd, J = 7.4 Hz, 1.6 Hz), 6.860−6.803 (4H, m), 5.645 (1H, t, J = 5.6 Hz), 3.958 (2H, t, J = 6.6 Hz), 3.827−3.749 (4H, m), 3.538−3.478 (1H, m), 3.173−3.055 (4H, m), 2.892 (2H, t, J = 7.6 Hz), 2.412 (2H, t, J = 7.6 Hz), 1.792 (2H, quin, J = 7.0 Hz), 1.490−1.418 (2H, m), 1.392−1.275 (6H, m), 0.889 (3H, t, J = 6.9 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): 174.00, 158.63, 156.80, 144.17, 144.12, 135.27, 130.29, 130.04, 128.99, 128.30, 127.88, 127.54, 127.01, 120.31, 113.17, 111.15, 86.47, 70.45, 67.91, 64.77, 55.17, 42.92, 36.51, 31.78, 29.35, 29.03, 26.73, 26.12, 22.60, 14.08. HRMS (ESI-TOF, $[M + Na]^+$): calcd for $C_{39}H_{47}NNaO_5^+$, 632.3346; found, 632.3322.

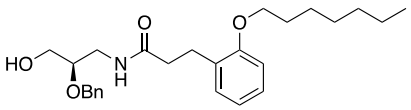
*Compound **66**.*



Compound **65** (1271.0 mg, 2.0085 mmol) was dissolved in anhydrous THF (10 mL). NaH (60% dispersion in oil, 119.1 mg) was added to the solution at 0 °C, and the mixture was stirred at 0 °C under an Ar atmosphere for 20 min. Benzyl bromide (310 μL) was added to the mixture, and the mixture was stirred at room temperature under an Ar atmosphere for 3.1 h. A saturated aqueous solution of $NaHCO_3$ (20 mL) was added, and the whole was extracted three times with AcOEt (20 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered, and the solvent was evaporated. The residue was column-chromatographed on an open column with silica gel twice (first, *n*-hexane/AcOEt = 3:1, second, *n*-hexane/AcOEt = 7:2) to afford **66** (1281.9 mg, 1.8315 mmol, 91%).

^{1}H NMR (400 MHz, $CDCl_3$): 7.451−7.427 (4H, m), 7.349−7.256 (11H, m), 7.229−7.187 (2H, m), 7.139 (1H, ddd, J = 7.8 Hz, 7.8 Hz, 1.7 Hz), 7.092 (1H, dd, J = 7.4 Hz, 1.6 Hz), 6.843−6.782 (4H, m), 5.651−5.635 (1H, m), 4.596 (1H, d, J = 11.7 Hz), 4.412 (1H, d, J = 11.7 Hz), 3.919 (2H, t, J = 6.6 Hz), 3.764 (3H, s), 3.638−3.552 (2H, m), 3.295−3.140 (3H, m), 2.879 (2H, t, J = 7.7 Hz), 2.353 (2H, t, J = 7.7 Hz), 1.760 (2H, quin, J = 7.0 Hz), 1.462−1.390 (2H, m), 1.365−1276 (6H, m), 0.880 (3H, t, J = 6.9 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): 172.59, 158.56, 156.80, 144.24, 144.22, 138.25, 135.40, 130.31, 129.92, 129.20, 128.44, 128.37, 127.84, 127.75, 127.40, 126.91, 120.24, 113.12, 111.07, 86.49, 71.88, 67.84, 63.70, 55.17, 40.64, 36.59, 31.77, 29.31, 29.01, 26.62, 26.08, 22.60, 14.07. HRMS (ESI-TOF, $[M + H]^+$): calcd for $C_{46}H_{53}NNaO_5^+$, 722.3816; found, 722.3787.

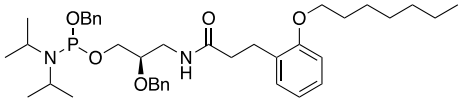
*Compound **67**.*



Compound **66** (1249.1 mg, 1.7846 mmol) was dissolved in CH_2Cl_2 (2.5 mL) and MeOH (7.5 mL). TsOH·H_2O (34.3 mg, 0.180 mmol) was added, and the whole was stirred at room temperature for 2 h. Et_3N (75 μL) was added, and the solvent was evaporated. The residue was column-chromatographed on an open column with silica gel (*n*-hexane/AcOEt = 2:1 to *n*-hexane/acetone = 2:1) to afford **67** as a colorless solid (745.1 mg, 1.743 mmol, 98%).

^{1}H NMR (400 MHz, $CDCl_3$): 7.366−7.269 (5H, m), 7.184−7.118 (2H, m), 6.846 (1H, ddd, J = 7.4 Hz, 7.4 Hz, 1.0 Hz), 6.808 (1H, d, J = 8.1 Hz), 5.757 (1H, t, J = 5.7 Hz), 4.563−4.481 (2H, m), 3.938 (2H, t, J = 6.5 Hz), 3.656−3.594 (1H, m), 3.533−3.464 (2H, m), 3.407−3.340 (1H, m), 3.303−3.242 (1H, m), 3.224−3.190 (1H, m), 2.941 (2H, t, J = 7.5 Hz), 2.506 (2H, t, J = 7.4 Hz), 1.783 (2H, quin, J = 7.0 Hz), 1.480−1.409 (2H, m), 1.384−1.273 (6H, m), 0.889 (3H, t, J = 6.9 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): 174.19, 156.78, 138.04, 130.12, 128.76, 128.52, 127.94, 127.75, 127.61, 120.36, 111.15, 77.59, 71.64, 67.86, 60.55, 39.30, 36.49, 31.78, 29.34, 29.02, 26.66, 26.12, 22.60, 14.07. HRMS (ESI-TOF, $[M + H]^+$): calcd for $C_{26}H_{38}NO_4^+$, 428.2795; found, 428.2787.

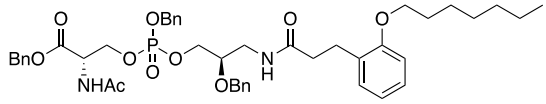
*Compound **68**.*



Compound **67** (300.2 mg, 0.7022 mmol) was dissolved in CH_2Cl_2 and toluene. To remove traces of water, the solution was evaporated. Compound **3** (354.2 mg, 1.046 mmol) and anhydrous CH_2Cl_2 (2.5 mL) were added. A solution of 1*H*-tetrazole (51.8 mg, 0.739 mmol) in anhydrous THF (2.5 mL) was added at 0 °C. The mixture was stirred at room temperature under an Ar atmosphere for 3.5 h. A saturated aqueous solution of $NaHCO_3$ (10 mL) was added, and the whole was extracted three times with CH_2Cl_2 (10 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated to afford a colorless oil. The residue was column-chromatographed on an open column with silica gel (*n*-hexane/AcOEt/Et_3N = 40:1:1 to 40:10:1) to afford **68** as a colorless oil (251.9 mg, 0.3789 mmol, 54%).

HRMS (ESI-TOF, $[M + H]^+$): calcd for $C_{39}H_{58}N_2O_5P^+$, 665.4078; found, 665.4072.

*Compound **69**.*



Compounds **5** (45.9 mg, 0.193 mmol) and **68** (229.6 mg, 0.3453 mmol) were dissolved in CH_2Cl_2 and toluene. To remove traces of water, the solution was evaporated. The mixture was dissolved in anhydrous CH_2Cl_2 (1.0 mL). A solution of 1*H*-tetrazole (28.3 mg, 0.404 mmol) in anhydrous THF (1.0 mL) was added at 0 °C. The mixture was stirred at room temperature under an Ar atmosphere for 3.5 h. TBHP in decane (5.0−6.0 M) (73 μL) was added, and the mixture was stirred at room temperature for 1 h. H_2O (10 mL) was added, and the whole was extracted three times with CH_2Cl_2 (10 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated to afford a colorless oil. The residue was column-chromatographed on a flash column with silica gel ($CHCl_3$/AcOEt = 1:1 to *n*-hexane/acetone = 1:1) to afford **69** as a colorless oil (127.4 mg, 0.1559 mmol, 81%).

^{1}H NMR (400 MHz, $CDCl_3$): (mixtures of diastereomers) 7.380−7.284 (15H, m), 7.166−7.086 (2H, m), 6.932 (J = 7.9 Hz) and 6.881 (J = 7.8 Hz) (1H, 2d), 6.842−6.777 (2H, m), 5.841 (J = 5.9 Hz) and 5.785 (J = 5.8 Hz) (1H, 2t), 5.211−5.112 (2H, m), 5.000−4.969 (2H, m), 4.852−4.790 (1H, m), 4.595−4.561 (1H, m), 4.457−4.384 (2H, m), 4.295−4.207 (1H, m), 4.014−3.798 (4H, m), 3.590−3.532 (1H, m), 3.403−3.278 (2H, m), 2.931−2.894 (2H, m), 2.460−2.406 (2H, m), 1.989 and 1.964 (3H, 2s), 1.775 (2H, quin, J = 7.0 Hz), 1.472−1.400 (2H, m), 1.375−1.277 (6H, m), 0.884 (3H, t, J = 6.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): (mixtures of diastereomers) 173.00, 172.94, 170.29, 170.25, 168.80, 168.78, 156.80, 137.62, 137.59, 135.40, 135.33, 135.00, 129.99, 128.97, 128.80, 128.77, 128.67, 128.60, 128.52, 128.50, 128.28, 128.20, 128.03, 128.00, 127.99, 127.95, 127.92, 127.63, 127.49, 120.25, 120.23, 111.11, 75.72, 75.65, 75.58, 72.02, 72.00, 69.80, 69.74, 67.85, 67.77, 67.64, 67.60, 67.53, 67.48, 67.37, 67.32, 66.69, 66.63, 66.55, 66.49, 52.78, 52.72, 39.31, 39.27, 36.42, 36.40, 31.76, 29.31,

https://doi.org/10.1021/acs.jmedchem.1c00347
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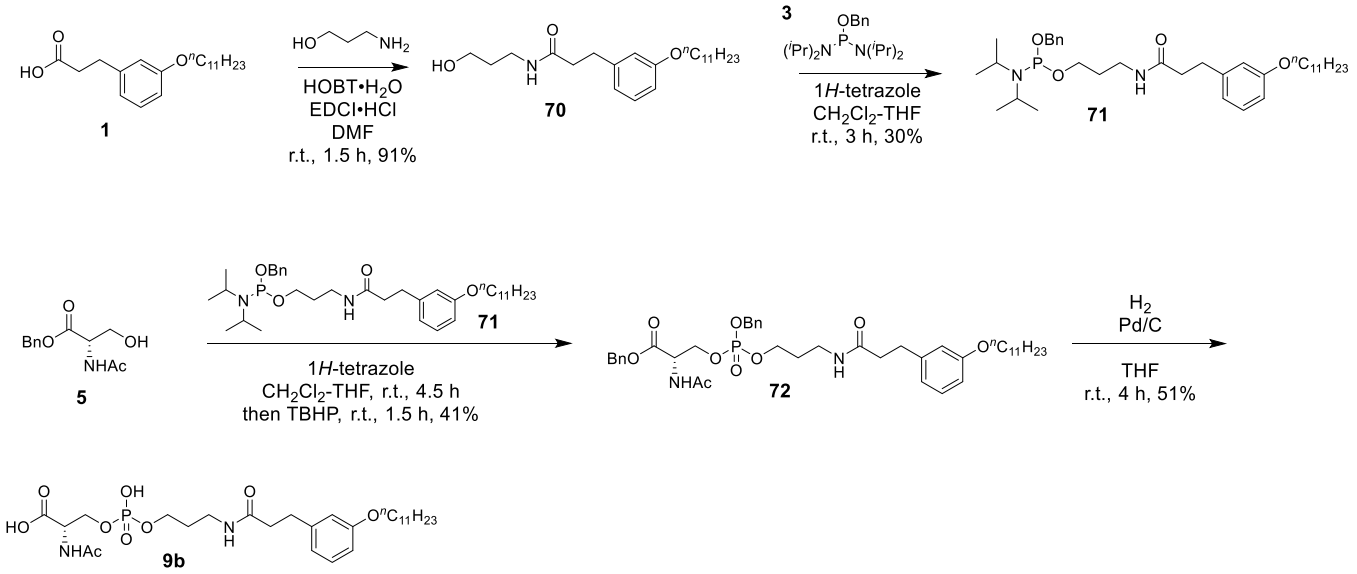
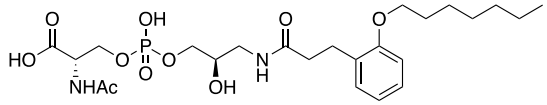


Figure 22. Synthesis of **9b**.

29.00, 26.59, 26.56, 26.07, 23.10, 22.84, 22.58, 14.06. HRMS (ESI-TOF, $[M + Na]^+$): calcd for $C_{45}H_{57}N_2NaO_{10}P^+$, 839.3643; found, 839.3649.

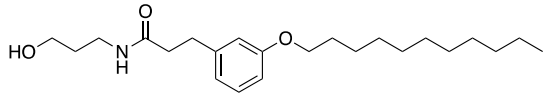
*Compound **9a**.*



Compound **69** (90.8 mg, 0.111 mmol) and Pd/C (18.7 mg) were dissolved in THF (6.0 mL). The mixture was stirred at room temperature under a H_2 atmosphere for 5.5 h. The mixture was filtered on Celite, and the solvent was evaporated. The residue was purified by reversed-phase medium-pressure liquid chromatography (CH_3CN/H_2O = 3:7 to 7:3, containing 0.1% HCOOH) and then by flash column chromatography ($CHCl_3$/acetone/AcOH = 4:1:1 to 1:1:1) to afford **9a** as a colorless powder (17.8 mg, 0.0326 mmol, 29%).

^{1}H NMR (400 MHz, DMSO-d_6): 7.902 (1H, t, J = 5.5 Hz), 7.150−7.098 (2H, m), 6.900 (1H, d, J = 7.8 Hz), 6.817 (1H, dd, J = 7.3 Hz, 7.3 Hz), 4.247 (1H, br s), 4.063 (1H, br s), 3.947 (2H, t, J = 6.4 Hz), 3.847 (1H, br s), 3.655−3.506 (3H, m), 3.151−3.119 (1H, m), 3.049−2.983 (1H, m), 2.756 (2H, t, J = 7.8 Hz), 2.336 (2H, t, J = 7.8 Hz), 1.850 (3H, s), 1.726 (2H, quin, J = 6.9 Hz), 1.463−1.391 (2H, m), 1.365−1.210 (6H, m), 0.864 (3H, t, J = 6.9 Hz). ^{31}P NMR (161 MHz, DMSO-d_6): −0.13. HRMS (ESI-TOF, $[M - H]^-$): calcd for $C_{24}H_{38}N_2O_{10}P^-$, 545.2270; found, 545.2298. HPLC: 94% (CH_3CN/H_2O = 4:6 (0.1% HCO_2H)).

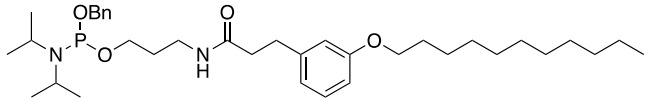
Synthesis of 9b (Figure 22). *Compound **70**.*



Compound **1** (475.2 mg, 1.483 mmol), 3-amino-1-propanol (124.1 mg, 1.652 mmol), and HOBT·H_2O (239.5 mg, 1.772 mmol) were dissolved in anhydrous DMF (5.0 mL). EDCI·HCl (342.2 mg, 1.785 mmol) was added at 0 °C, and the whole was stirred at room temperature under an Ar atmosphere for 1.5 h. A saturated aqueous solution of $NaHCO_3$ (10 mL) was added, and the precipitate **70** was obtained by filtration (colorless powder, 509.0 mg, 1.348 mmol, 91%) (Figure 22).

^{1}H NMR (400 MHz, $CDCl_3$): 7.205−7.164 (1H, m), 6.777−6.725 (3H, m), 5.741 (1H, br s), 3.927 (2H, t, J = 6.6 Hz), 3.527 (2H, t, J = 5.6 Hz), 3.368 (2H, q, J = 6.1 Hz), 2.932 (2H, t, J = 7.6 Hz), 2.488 (2H, t, J = 7.6 Hz), 1.763 (2H, quin, J = 7.0 Hz), 1.627−1.574 (2H, m), 1.569−1.404 (2H, m), 1.358−1.266 (14H, m), 0.881 (3H, t, J = 6.9 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): 173.31, 159.32, 142.21, 129.48, 120.49, 114.69, 112.21, 67.93, 59.19, 38.38, 36.24, 32.19, 31.89, 31.78, 29.59, 29.57, 29.39, 29.31, 29.30, 26.05, 22.66, 14.09. HRMS (ESI-TOF, $[M + H]^+$): calcd for $C_{23}H_{40}NO_3^+$, 378.3003; found, 378.2999.

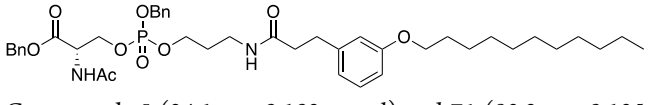
*Compound **71**.*



Compound **70** (226.0 mg, 0.5986 mmol) was dissolved in CH_2Cl_2 and toluene. To remove traces of water, the solution was evaporated. Compound **3** (303.9 mg, 0.8978 mmol) and anhydrous CH_2Cl_2 (2.0 mL) were added. A solution of 1*H*-tetrazole (44.5 mg, 0.635 mmol) in anhydrous THF (2.0 mL) was added at 0 °C. The mixture was stirred at room temperature under an Ar atmosphere for 3 h. A saturated aqueous solution of $NaHCO_3$ (10 mL) was added, and the whole was extracted three times with CH_2Cl_2 (10 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated to afford a colorless oil. The residue was column-chromatographed on an open column with silica gel (*n*-hexane/AcOEt/Et_3N = 40:1:1 to 80:40:3) to afford **71** as a colorless oil (112.2 mg, 0.1825 mmol, 30%).

HRMS (ESI-TOF, $[M + H]^+$): calcd for $C_{36}H_{60}N_2O_4P^+$, 615.4285; found, 615.4256.

*Compound **72**.*



Compounds **5** (24.1 mg, 0.102 mmol) and **71** (83.2 mg, 0.135 mmol) were dissolved in CH_2Cl_2 and toluene. To remove traces of water, the solution was evaporated. The mixture was dissolved in anhydrous CH_2Cl_2 (0.4 mL). A solution of 1*H*-tetrazole (14.5 mg, 0.207 mmol) in anhydrous THF (0.4 mL) was added at 0 °C. The mixture was stirred at room temperature under an Ar atmosphere for 4.5 h. TBHP in decane (5.0−6.0 M) (36 μL) was added, and the mixture was stirred at room temperature for 1.5

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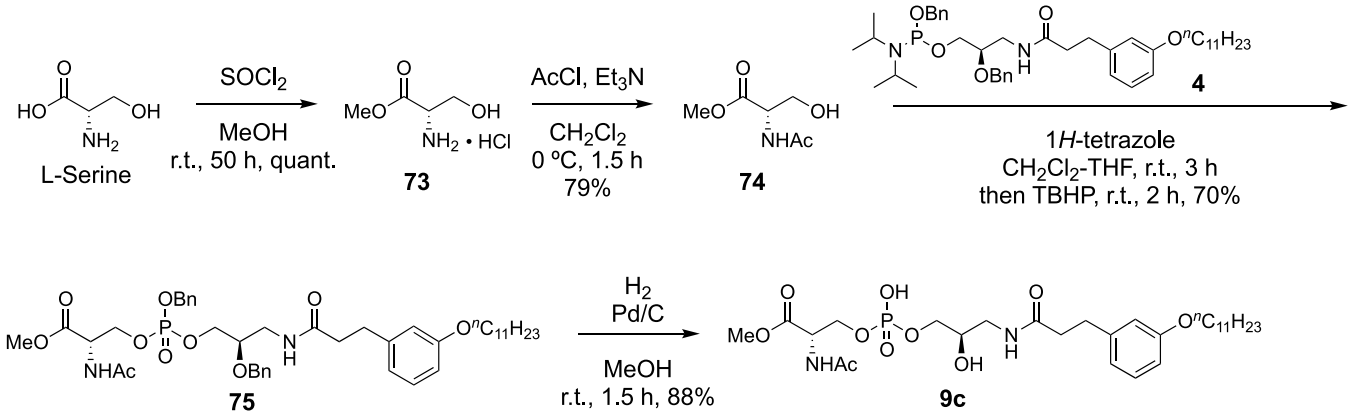
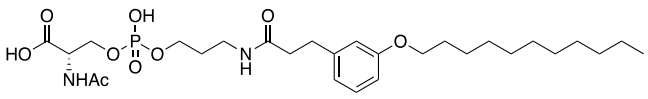


Figure 23. Synthesis of **9c**.

h. H_2O (10 mL) was added, and the whole was extracted three times with CH_2Cl_2 (10 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated to afford a colorless oil. The residue was column-chromatographed on a flash column with silica gel ($CHCl_3$/AcOEt = 1:1 to *n*-hexane/acetone = 3:2 to 0:1) to afford **72** as a colorless oil (31.8 mg, 0.0415 mmol, 41%).

^{1}H NMR (400 MHz, $CDCl_3$): (mixtures of diastereomers) 7.384−7.290 (10H, m), 7.154 (1H, dd, *J* = 7.6 Hz, 7.6 Hz), 6.893 (0.5H, d, *J* = 7.7 Hz), 6.757−6.704 (3.5H, m), 6.086−6.026 (1H, m), 5.213−5.132 (2H, m), 5.028−4.934 (2H, m), 4.855−4.794 (1H, m), 4.450−4.346 (1H, m), 4.276−4.221 (1H, m), 3.922−3.844 (4H, m), 3.281−3.187 (2H, m), 2.901 (2H, t, *J* = 7.7 Hz), 2.431 (2H, t, *J* = 7.7 Hz), 2.005 (3H, d, *J* = 7.4 Hz), 1.783−1.682 (4H, m), 1.464−1.394 (2H, m), 1.350−1.266 (14H, m), 0.880 (3H, t, *J* = 6.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): (mixtures of diastereomers) 172.38, 172.36, 170.19, 170.18, 168.89, 168.83, 159.26, 142.38, 135.41, 135.35, 135.29, 134.89, 134.88, 129.37, 128.88, 128.82, 128.71, 128.70, 128.62, 128.57, 128.54, 128.27, 128.18, 128.05, 127.97, 120.45, 120.43, 114.70, 112.05, 69.82, 69.77, 69.74, 69.68, 67.87, 67.73, 67.67, 67.25, 67.23, 67.20, 67.17, 65.63, 65.57, 65.48, 65.42, 52.85, 52.80, 52.74, 38.23, 35.34, 35.30, 31.87, 31.72, 29.76, 29.73, 29.70, 29.68, 29.58, 29.55, 29.39, 29.29, 26.03, 22.92, 22.64, 14.08. HRMS (ESI-TOF, [M + Na]$^+$): calcd for $C_{42}H_{59}N_2NaO_9P^+$, 789.3850; found, 789.3848.

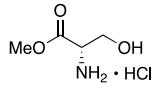
Compound ***9b***.



Compound **72** (31.6 mg, 0.0412 mmol) and Pd/C (13.3 mg) were dissolved in THF (5 mL). The mixture was stirred at room temperature under a H_2 atmosphere for 4 h. The mixture was filtered on Celite, and the solvent was evaporated. The residue was purified by reversed-phase medium-pressure liquid chromatography (CH_3CN/H_2O = 3:7 to 7:3, containing 0.1% HCOOH) to afford **9b** as a yellow sticky solid (12.3 mg, 0.0210 mmol, 51%).

^{1}H NMR (400 MHz, DMSO-d_6): 8.544 (1H, br s), 7.913 (1H, br s), 7.143 (1H, dd, *J* = 8.0 Hz, 8.0 Hz), 6.744−6.694 (3H, m), 4.434−4.418 (1H, m), 4.000−3.890 (4H, m), 3.768−3.735 (2H, m), 3.085 (2H, q, *J* = 6.2 Hz), 2.756 (2H, t, *J* = 7.8 Hz), 2.335 (2H, t, *J* = 7.8 Hz), 1.851 (3H, s), 1.712−1.611 (4H, m), 1.388−1.353 (2H, m), 1.245 (14H, br s), 0.851 (3H, t, *J* = 6.8 Hz). HRMS (ESI-TOF, [M − H]$^-$): calcd for $C_{28}H_{46}N_2O_9P^-$, 585.2946; found, 585.2960. HPLC: >99% (CH_3CN/H_2O = 3:2 (0.1% HCO_2H)).

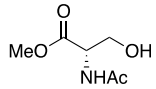
Synthesis of 9c (Figure 23). *Compound* ***73***.



L-Serine (526.7 mg, 5.012 mmol) was suspended in anhydrous MeOH (12.5 mL). $SOCl_2$ (1.1 mL, 15.1 mmol) was added dropwise, and the mixture was stirred at room temperature for 50 h. The whole was evaporated and dried to afford **73** as a colorless solid (782.4 mg, 5.029 mmol as HCl salt, quant.) (Figure 23).

^{1}H NMR (400 MHz, MeOD-d_4): 4.135 (1H, t, *J* = 4.0 Hz), 4.008 (1H, dd, *J* = 11.8 Hz, 4.5 Hz), 3.924 (1H, dd, *J* = 11.8 Hz, 3.5 Hz), 3.855 (3H, s). ^{13}C NMR (100 MHz, MeOD-d_4): 169.36, 60.66, 56.08, 53.67. HRMS (ESI-TOF, [M + H]$^+$): calcd for $C_4H_{10}NO_3^+$, 120.0655; found, 120.0676.

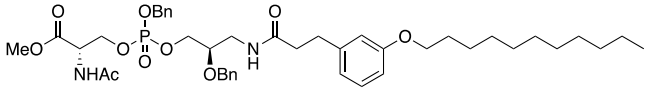
Compound ***74***.



Compound **73** (389.9 mg, 2.506 mmol) was dissolved in anhydrous CH_2Cl_2 (10 mL). Et_3N (730 μL) was added. Acetyl chloride (190 μL, 2.68 mmol) was added at 0 °C, and the mixture was stirred at 0 °C under an Ar atmosphere for 1.5 h. MeOH (ca. 2 mL) was added, and the solvent was evaporated. The residue was column-chromatographed on a flash column with silica gel twice (first, $CHCl_3$/MeOH = 2:1, second, $CHCl_3$/MeOH = 10:1) to afford **74** as a colorless oil (318.2 mg, 1.974 mmol, 79%).

^{1}H NMR (400 MHz, DMSO-d_6): 8.178 (1H, d, *J* = 7.6 Hz), 5.020 (1H, t, *J* = 5.7 Hz), 4.349−4.304 (1H, m), 3.696−3.564 (5H, m), 1.866 (3H, s). ^{13}C NMR (100 MHz, DMSO-d_6): 171.23, 169.43, 61.27, 54.63, 51.77, 22.31. HRMS (ESI-TOF, [M + H]$^+$): calcd for $C_6H_{11}NNaO_4^+$, 184.0580; found, 184.0575.

Compound ***75***.



Compounds **74** (26.4 mg, 0.164 mmol) and **4** (201.7 mg, 0.2798 mmol) were dissolved in CH_2Cl_2 and toluene. To remove traces of water, the solution was evaporated. The mixture was dissolved in anhydrous CH_2Cl_2 (0.75 mL). A solution of 1*H*-tetrazole (21.9 mg, 0.313 mmol) in anhydrous THF (0.75 mL) was added at 0 °C. The mixture was stirred at room temperature under an Ar atmosphere for 3 h. TBHP in decane (5.0−6.0 M) (58 μL)

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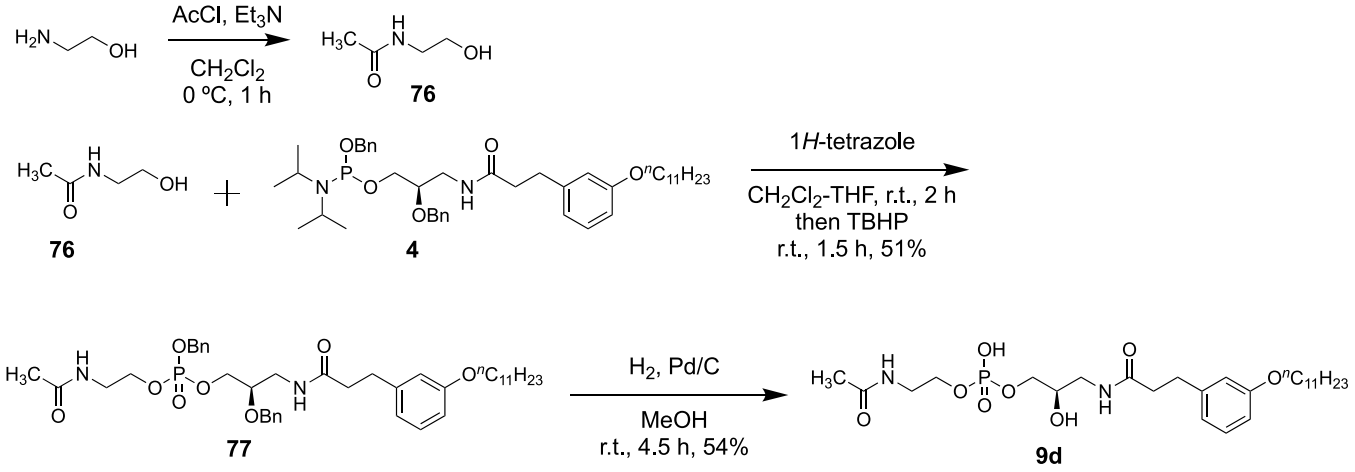
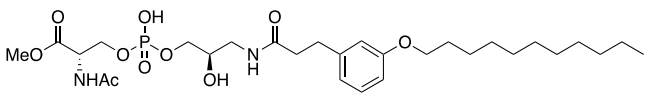


Figure 24. Synthesis of **9d**.

was added, and the mixture was stirred at room temperature for 2 h. H_2O (10 mL) was added, and the whole was extracted three times with CH_2Cl_2 (10 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated to afford a colorless oil. The residue was column-chromatographed on a flash column with silica gel ($CHCl_3$/AcOEt = 1:1 to *n*-hexane/acetone = 1:1) to afford **75** as a colorless oil (92.0 mg, 0.115 mmol, 70%).

^{1}H NMR (400 MHz, $CDCl_3$): (mixtures of diastereomers) 7.380−7.276 (10H, m), 7.181−7.133 (1H, m), 6.856−6.806 (1H, m), 6.750−6.705 (3H, m), 5.934 and 5.858 (1H, 2t, J = 5.9 Hz), 5.065−5.025 (2H, m), 4.807−4.759 (1H, m), 4.604 (1H, d, J = 11.6 Hz), 4.464 (1H, dd, J = 11.6 Hz, 6.7 Hz), 4.430−4.363 (1H, m), 4.285−4.200 (1H, m), 4.069−3.877 (4H, m), 3.732 and 3.712 (3H, 2s), 3.650−3.571 (1H, m), 3.398−3.368 (2H, m), 2.902−2.858 (2H, m), 2.483−2.349 (2H, m), 1.994 and 1.976 (3H, 2s), 1.784−1.714 (2H, m), 1.467−1.396 (2H, m), 1.351−1.254 (14H, m), 0.880 (3H, t, J = 6.9 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): (mixtures of diastereomers) 172.42, 172.37, 170.21, 169.39, 159.30, 142.28, 137.59, 137.56, 135.38, 135.33, 129.42, 128.85, 128.83, 128.71, 128.57, 128.55, 128.10, 128.07, 128.05, 128.01, 120.42, 114.70, 112.06, 75.60, 75.53, 72.05, 72.03, 69.88, 69.80, 67.88, 67.53, 67.47, 67.40, 67.34, 66.70, 66.64, 66.51, 66.45, 52.80, 52.63, 52.59, 39.18, 38.13, 31.88, 31.60, 29.59, 29.57, 29.41, 29.31, 26.06, 22.88, 22.66, 14.10. HRMS (ESI-TOF, [M + Na]$^+$): calcd for $C_{43}H_{61}N_2NaO_{10}P^+$, 819.3956; found, 819.3932.

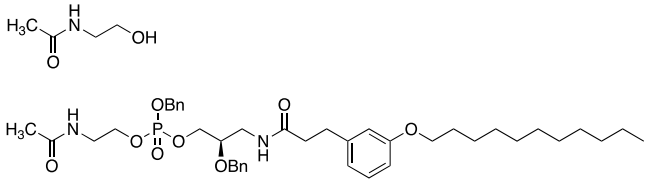
Compound ***9c****.*



Compound **75** (74.8 mg, 0.0939 mmol) and Pd/C (18.1 mg) were dissolved in MeOH (5 mL). The mixture was stirred at room temperature under a H_2 atmosphere for 1.5 h. The mixture was filtered on Celite, and the solvent was evaporated. The residue was purified by a flash column with silica gel ($CHCl_3$/MeOH/AcOH = 8:1:1 to 7:2:1) to afford **9c** as a colorless powder (51.1 mg, 0.0829 mmol, 88%).

^{1}H NMR (400 MHz, DMSO-d_6): 9.018 (1H, br s), 7.966 (1H, t, J = 5.7 Hz), 7.143 (1H, dd, J = 8.1 Hz, 8.1 Hz), 6.751−6.694 (3H, m), 5.727 (1H, br s), 4.354−4.338 (1H, m), 3.936−3.892 (4H, m), 3.621−3.528 (6H, m), 3.164−3.104 (1H, m), 3.044−2.981 (1H, m), 2.753 (2H, t, J = 7.9 Hz), 2.363 (2H, t, J = 7.9 Hz), 1.840 (3H, s), 1.680 (2H, quin, J = 6.9 Hz), 1.404−1.354 (2H, m), 1.246 (14H, br s), 0.852 (3H, t, J = 6.8 Hz). HRMS (ESI-TOF, [M − H]$^-$): calcd for $C_{29}H_{48}N_2O_{10}P^-$, 615.3052; found, 615.3075. HPLC: >99% (CH_3CN/H_2O = 3:2 (0.1% HCO_2H)).

Synthesis of 9d (Figure 24). *Compounds* ***76*** *and* ***77****.*



2-Aminoethanol (61.4 mg, 1.01 mmol) was dissolved in anhydrous CH_2Cl_2 (3.0 mL). Et_3N (170 μL, 2.3 mmol) was added. AcCl (80 μL, 0.92 mmol) was added at 0 °C, and the mixture was stirred at 0 °C for 1 h. The solvent was evaporated. The residue and **4** (178.2 mg, 0.2472 mmol) were dissolved in CH_2Cl_2 and toluene. To remove traces of water, the solution was evaporated. The mixture was dissolved in anhydrous CH_2Cl_2 (2.0 mL). A solution of 1*H*-tetrazole (35.4 mg, 0.505 mmol) in anhydrous THF (2.0 mL) was added at 0 °C. The mixture was stirred at room temperature under an Ar atmosphere for 2 h. TBHP in decane (5.0−6.0 M) (91 μL) was added, and the mixture was stirred at room temperature for 1.5 h. H_2O (10 mL) was added, and the whole was extracted three times with CH_2Cl_2 (10 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated. The residue was column-chromatographed on a flash column with silica gel ($CHCl_3$/AcOEt = 1:1 to *n*-hexane/acetone = 1:2) to afford **77** as a colorless oil (93.2 mg, 0.126 mmol, 51% from **4**) (Figure 24).

^{1}H NMR (400 MHz, $CDCl_3$): (mixture of diastereomers) 7.378−7.273 (10H, m), 7.180−7.132 (1H, m), 6.742−6.703 (3H, m), 6.426 (1H, br s), 5.944 and 5.853 (1H, 2t, J = 5.8 Hz), 5.078−5.045 (2H, m), 4.591 (1H, d, J = 11.6 Hz), 4.461 (1H, dd, J = 11.6 Hz, 7.8 Hz), 4.074−3.883 (6H, m), 3.656−3.573 (1H, m), 3.494−3.314 (4H, m), 2.893−2.850 (2H, m), 2.417−2.379 (2H, m), 1.919 (3H, s), 1.747 (2H, quin, J = 6.8 Hz), 1.444−1.393 (2H, m), 1.314−1.261 (14H, m), 0.877 (3H, t, J = 6.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): (mixture of diastereomers) 172.38, 170.49, 159.31, 142.18, 137.54, 135.50, 135.45, 129.45, 128.84, 128.71, 128.57, 128.13, 128.09, 128.00, 120.40, 114.70, 112.08, 75.66, 75.60, 72.09, 72.05, 69.74, 67.89, 67.14, 67.08, 67.04, 66.98, 66.46, 66.39, 66.29, 66.23, 39.81, 39.75, 39.39, 38.13, 31.88, 31.60, 29.59, 29.57, 29.41, 29.31, 26.06, 23.03, 22.66, 14.10. HRMS (ESI-TOF, [M + Na]$^+$): calcd for $C_{41}H_{59}N_2NaO_8P^+$, 761.3901; found, 761.3881. Anal. Calcd for $C_{41}H_{59}N_2O_8P{\cdot}0.5H_2O$: C, 65.84; H, 8.09; N, 3.75. Found: C, 65.79; H, 7.97; N, 4.09.

https://doi.org/10.1021/acs.jmedchem.1c00347
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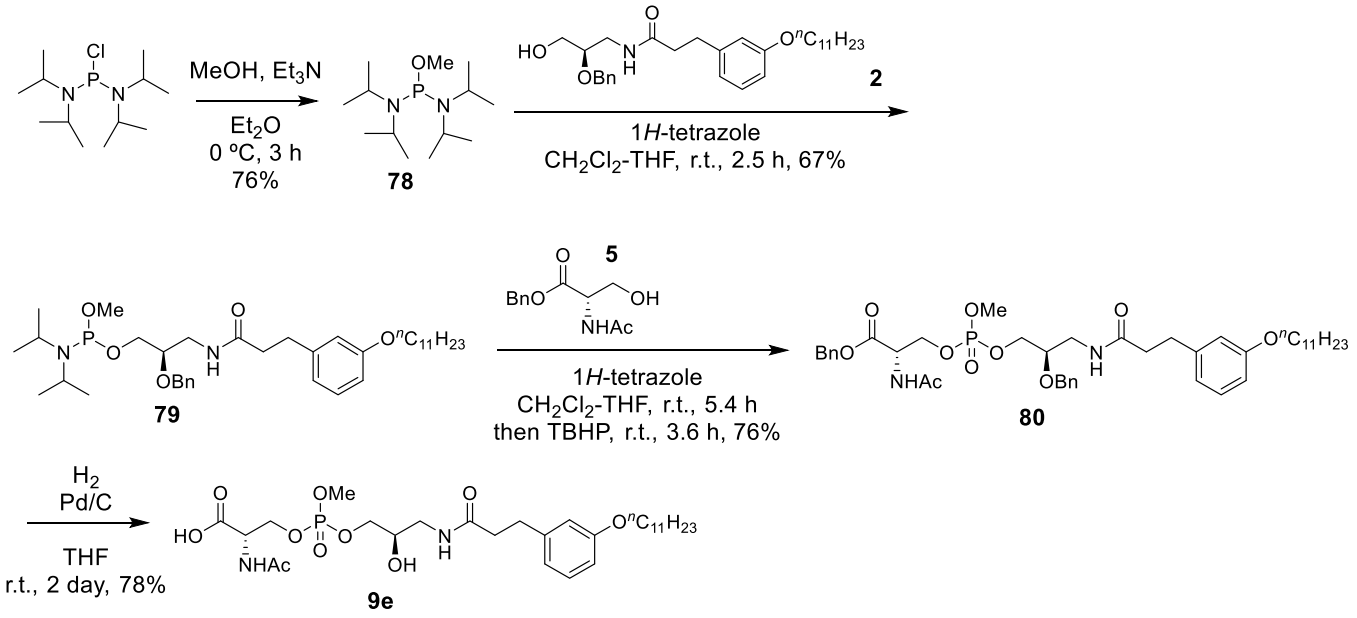
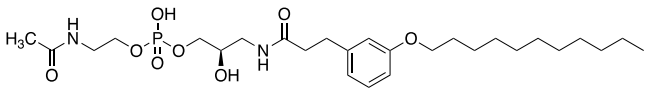


Figure 25. Synthesis of **9e**.

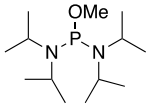
*Compound **9d**.*



Compound **77** (73.3 mg, 0.0992 mmol) and Pd/C (24.9 mg) were dissolved in MeOH (5.0 mL). The mixture was stirred at room temperature under a H_2 atmosphere for 4.5 h. The mixture was filtered on Celite, and the solvent was evaporated. The residue was purified by reversed-phase medium-pressure liquid chromatography (CH_3CN/H_2O = 3:7 to 7:3, containing 0.1% HCOOH) to afford **9d** as a colorless powder (29.7 mg, 0.0532 mmol, 54%).

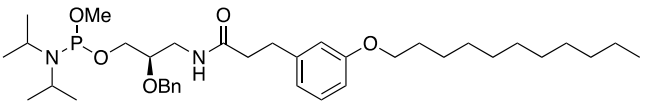
^{1}H NMR (400 MHz, DMSO-d_6): 8.048 (1H, t, J = 4.8 Hz), 7.897 (1H, t, J = 5.7 Hz), 7.148 (1H, dd, J = 8.1 Hz, 8.1 Hz), 6.753−6.699 (3H, m), 3.910 (2H, t, J = 6.5 Hz), 3.824 (2H, q, J = 6.3 Hz), 3.772−3.677 (2H, m), 3.660−3.602 (1H, m), 3.241 (2H, q, J = 5.7 Hz), 3.220−3.124 (1H, m), 3.057−2.993 (1H, m), 2.757 (2H, t, J = 7.9 Hz), 2.376 (2H, t, J = 7.9 Hz), 1.806 and 1.788 (3H, 2s), 1.683 (2H, quin, J = 6.9 Hz), 1.406−1.356 (2H, m), 1.247 (14H, br s), 0.854 (3H, t, J = 6.9 Hz). HRMS (ESI-TOF, [M − H]$^-$): calcd for $C_{27}H_{46}N_2O_8P^-$, 557.2997; found, 557.3025. Anal. Calcd for $C_{27}H_{47}N_2O_8P{\cdot}0.5H_2O$: C, 57.13; H, 8.52; N, 4.93. Found: C, 57.02; H, 8.26; N, 5.11. HPLC: 99% (CH_3CN/H_2O = 1:1 (0.1% HCO_2H)).

Synthesis of 9e (Figure 25). *Compound **78**.*



Bis(diisopropylamino)chlorophosphine (1080.7 mg, 4.0507 mmol) was dissolved in anhydrous Et_2O (5.0 mL). A solution of Et_3N (565 μL) and anhydrous MeOH (180 μL) in anhydrous Et_2O (2.5 mL) was added dropwise at 0 °C. The flask contained the solution was washed with additional anhydrous Et_2O (2.5 mL), and the solvent was added to the reaction mixture. The whole was stirred at 0 °C under an Ar atmosphere for 3 h. The mixture was filtered on Celite, and the solvent was evaporated to afford **78** as a colorless oil (812.7 mg, 3.097 mmol, 76%), which was used without further purification (Figure 25).

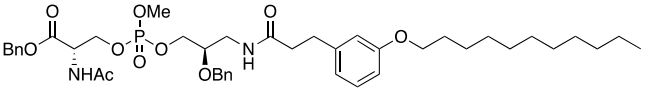
*Compound **79**.*



Compound **2** (485.7 mg, 1.004 mmol) was dissolved in CH_2Cl_2 and toluene. To remove traces of water, the solution was evaporated. Compound **78** (421.0 mg, 1.605 mmol) and anhydrous CH_2Cl_2 (4.0 mL) were added. A solution of 1*H*-tetrazole (74.3 mg, 1.06 mmol) in anhydrous THF (4.0 mL) was added at 0 °C. The mixture was stirred at room temperature under an Ar atmosphere for 2.5 h. A saturated aqueous solution of $NaHCO_3$ (15 mL) was added, and the whole was extracted three times with CH_2Cl_2 (15 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated to afford a colorless oil. The residue was column-chromatographed on an open column with silica gel (*n*-hexane/AcOEt/Et_3N = 40:10:1) to afford **79** as a colorless oil (437.0 mg, 0.6776 mmol, 67%).

^{1}H NMR (400 MHz, $CDCl_3$): 7.362−7.271 (5H, m), 7.186−7.145 (1H, m), 6.757−6.708 (3H, m), 5.819−5.792 (1H, m), 4.665 (1H, d, J = 11.8 Hz), 4.505 (1H, dd, J = 11.8 Hz, 7.0 Hz), 3.913 (2H, t, J = 6.6 Hz), 3.781−3.510 (6H, m), 3.415 and 3.382 (3H, 2d, J = 3.3 Hz), 3.341−3.204 (1H, m), 2.883 (2H, t, J = 7.8 Hz), 2.444−2.344 (2H, m), 1.759 (2H, quin, J = 7.0 Hz), 1.474−1.238 (16H, m), 1.189−1.165 (12H, m), 0.881 (3H, t, J = 6.9 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): 171.95, 159.29, 142.50, 142.47, 138.28, 129.40, 128.46, 127.96, 127.90, 127.81, 120.41, 114.60, 112.10, 71.88, 71.84, 67.86, 63.75, 63.58, 50.64, 50.54, 50.46, 50.37, 42.91, 42.79, 40.29, 40.24, 38.40, 31.89, 31.74, 29.60, 29.57, 29.41, 29.32, 26.06, 24.75, 24.68, 24.62, 22.67, 14.10. HRMS (ESI-TOF, [M + Na]$^+$): calcd for $C_{37}H_{61}N_2NaO_5P^+$, 667.4210; found, 667.4181.

*Compound **80**.*



Compounds **5** (47.2 mg, 0.199 mmol) and **79** (206.5 mg, 0.3202 mmol) were dissolved in CH_2Cl_2 and toluene. To remove traces of water, the solution was evaporated. The mixture was dissolved

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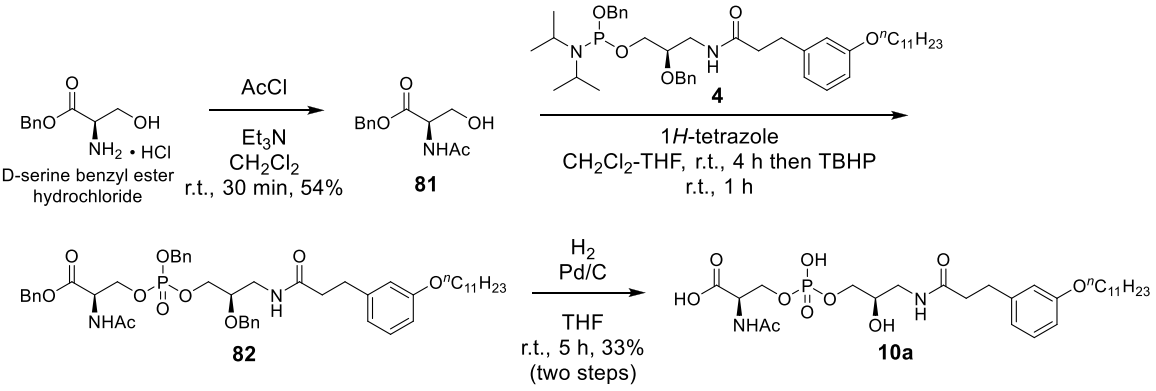
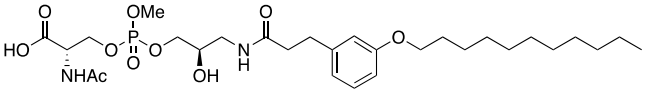


Figure 26. Synthesis of **10a**.

in anhydrous CH_2Cl_2 (1.0 mL). A solution of 1*H*-tetrazole (29.1 mg, 0.415 mmol) in anhydrous THF (1.0 mL) was added at 0 °C. The mixture was stirred at room temperature under an Ar atmosphere for 5.4 h. TBHP in decane (5.0−6.0 M) (73 μL) was added, and the mixture was stirred at room temperature for 3.6 h. H_2O (10 mL) was added, and the whole was extracted three times with CH_2Cl_2 (10 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated to afford a colorless oil. The residue was column-chromatographed on a flash column with silica gel ($CHCl_3$/AcOEt = 1:1 to *n*-hexane/acetone = 2:3) to afford **80** as a colorless oil (120.6 mg, 0.1513 mmol, 76%).

^{1}H NMR (400 MHz, $CDCl_3$): (mixtures of diastereomers) 7.369−7.296 (10H, m), 7.179−7.136 (1H, m), 6.942 (1H, d, *J* = 7.8 Hz), 6.752−6.709 (3H, m), 5.948−5.879 (1H, m), 4.049−3.889 (4H, m), 5.197 (2H, s), 4.883−4.817 (1H, m), 4.625 (1H, d, *J* = 11.6 Hz), 4.501−4.417 (2H, m), 4.354−4.254 (1H, m), 3.690 (*J* = 2.7 Hz) and 3.662 (*J* = 2.6 Hz) (3H, 2d), 3.647−3.598 (1H, m), 3.452−3.338 (2H, m), 2.883 (2H, t, *J* = 7.6 Hz), 2.494−2.356 (2H, m), 2.029 (3H, 2s), 1.786−1.732 (2H, m), 1.467−1.396 (2H, m), 1.352−1.264 (14H, m), 0.879 (3H, t, *J* = 6.9 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): (mixtures of diastereomers) 172.44, 172.38, 170.28, 170.27, 168.85, 168.81, 159.30, 142.27, 137.58, 137.55, 134.96, 129.42, 128.63, 128.58, 128.56, 128.54, 128.31, 128.26, 128.12, 128.09, 128.02, 120.43, 114.69, 112.07, 75.65, 75.58, 75.51, 72.08, 72.05, 67.88, 67.70, 67.69, 67.54, 67.49, 67.34, 67.29, 66.70, 66.64, 66.49, 66.43, 54.71, 54.63, 52.84, 52.78, 39.23, 38.14, 31.88, 31.60, 29.59, 29.56, 29.41, 29.31, 26.05, 22.93, 22.90, 22.66, 14.09. HRMS (ESI-TOF, $[M + Na]^+$): calcd for $C_{43}H_{61}N_2NaO_{10}P^+$, 819.3956; found, 819.3985.

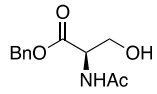
*Compound **9e**.*



Compound **80** (95.3 mg, 0.120 mmol) and Pd/C (21.6 mg) were dissolved in THF (5 mL). The mixture was stirred at room temperature under a H_2 atmosphere for 2 days. The mixture was filtered on Celite, and the solvent was evaporated. The residue was purified by a flash column with silica gel ($CHCl_3$/acetone/AcOH = 2:1:1) to afford **9e** as a colorless sticky oil (57.2 mg, 0.0928 mmol, 78%).

^{1}H NMR (400 MHz, DMSO-d_6): (mixtures of diastereomers) 8.054−7.968 (2H, m), 7.146 (1H, dd, *J* = 7.9 Hz, 7.9 Hz), 6.753−6.697 (3H, m), 4.369 (1H, br s), 4.257−4.204 (1H, m), 4.173−4.128 (1H, m), 3.909 (2H, t, *J* = 6.5 Hz), 3.875−3.747 (1.5H, m), 3.667−3.618 (3.5H, m), 3.162−3.042 (2H, m), 2.761 (2H, t, *J* = 7.8 Hz), 2.419−2.371 (2H, m), 1.879−1.859 (3H, m), 1.681 (2H, quin, *J* = 6.9 Hz), 1.390−1.246 (16H, m), 0.853 (3H, t, *J* = 6.8 Hz). HRMS (ESI-TOF, $[M - H]^-$): calcd for $C_{29}H_{48}N_2O_{10}P^-$, 615.3052; found, 615.3036. HPLC: 90% (CH_3CN/H_2O = 3:2 (0.1% HCO_2H)).

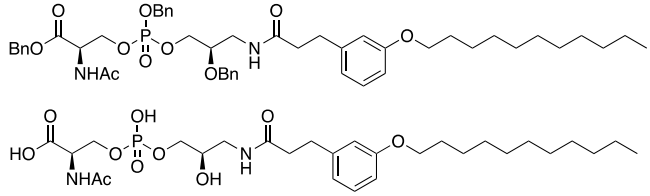
Synthesis of 10a (Figure 26). *Compound **81**.*



D-Serine benzyl ester hydrochloride (463.0 mg, 1.998 mmol) was dissolved in anhydrous CH_2Cl_2 (6.0 mL). Et_3N (700 μL) and acetyl chloride (312 μL, 4.40 mmol) were added, and the mixture was stirred at room temperature under an Ar atmosphere for 30 min. MeOH (ca. 2 mL) was added, and the solvent was evaporated with silica gel. The residue was column-chromatographed on an open column with silica gel (*n*-hexane/acetone = 2:1 to 1:1) to afford **81** as a colorless solid (258.1 mg, 1.088 mmol, 54%) (Figure 26).

^{1}H NMR (400 MHz, $CDCl_3$): 7.459−7.315 (5H, m), 6.486 (1H, d, *J* = 5.6 Hz), 5.219 (2H, s), 4.729−4.693 (1H, m), 4.020−3.968 (1H, m), 3.951−3.900 (1H, m), 2.556 (1H, t, *J* = 5.9 Hz), 2.057 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$): 170.66, 170.37, 135.06, 128.65, 128.54, 128.15, 67.53, 63.41, 54.86, 23.08. HRMS (ESI-TOF, $[M + Na]^+$): calcd for $C_{12}H_{15}NNaO_4^+$, 260.0893; found, 260.0889.

*Compounds **82** and **10a**.*



Compounds **81** (35.0 mg, 0.148 mmol) and **4** (172.5 mg, 0.2393 mmol) were dissolved in CH_2Cl_2 and toluene. To remove traces of water, the solution was evaporated. The mixture was dissolved in anhydrous CH_2Cl_2 (1.0 mL). A solution of 1*H*-tetrazole (20.8 mg, 0.297 mmol) in anhydrous THF (1.0 mL) was added at 0 °C. The mixture was stirred at room temperature under an Ar atmosphere for 4 h. TBHP in decane (5.0−6.0 M) (55 μL) was added, and the mixture was stirred at room temperature for 1 h. H_2O (10 mL) was added, and the whole was extracted three times with CH_2Cl_2 (10 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated to afford a colorless oil. The residue was column-chromatographed on a flash column with silica gel ($CHCl_3$/AcOEt = 1:1 to *n*-hexane/acetone = 2:3) to afford a mixture of **82** and **81** as a colorless oil (83.9 mg).

HRMS (ESI-TOF, $[M + NH_4]^+$): calcd for $C_{49}H_{69}N_3O_{10}P^+$, 890.4715; found, 890.4687.

The crude **82** (80.8 mg) and Pd/C (19.2 mg) were dissolved in THF (5.0 mL). The mixture was stirred at room temperature under a H_2

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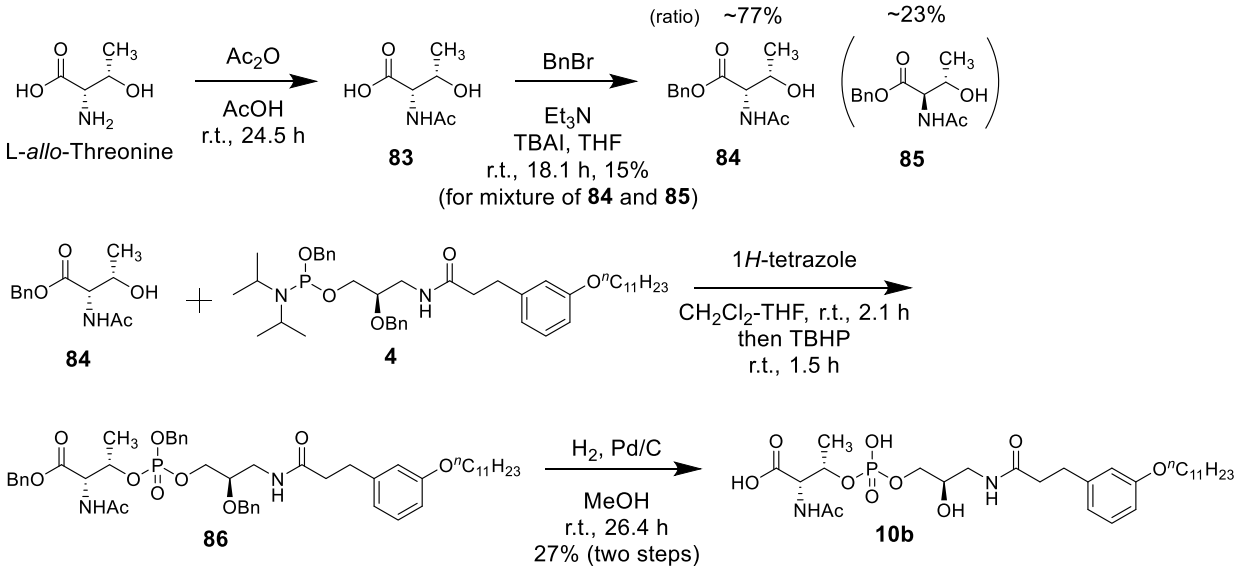
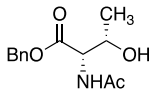


Figure 27. Synthesis of **10b**.

atmosphere for 5 h. The mixture was filtered on Celite, and the solvent was evaporated. The residue was purified by reversed-phase medium-pressure liquid chromatography (CH_3CN/H_2O = 3:7 to 7:3, containing 0.1% HCOOH) to afford **10a** as a colorless powder (29.8 mg, 0.0494 mmol, 33%).

^{1}H NMR (400 MHz, DMSO-d_6): 12.837 (1H, br s), 8.366 (1H, d, J = 7.6 Hz), 7.877 (1H, t, J = 5.7 Hz), 7.148 (1H, dd, J = 8.1 Hz, 8.1 Hz), 6.754−6.699 (3H, m), 4.487−4.445 (1H, m), 4.117−4.002 (2H, m), 3.911 (2H, t, J = 6.5 Hz), 3.766−3.686 (2H, m), 3.658−3.616 (1H, m), 3.191−3.130 (1H, m), 3.048−2.984 (1H, m), 2.758 (2H, t, J = 7.9 Hz), 2.378 (2H, t, J = 7.9 Hz), 1.870 and 1.862 (3H, 2s), 1.683 (2H, quin, J = 7.0 Hz), 1.406−1.248 (16H, m), 0.854 (3H, t, J = 6.8 Hz). HRMS (ESI-TOF, [M − H]$^-$): calcd for $C_{28}H_{46}N_2O_{10}P^-$, 601.2896; found, 601.2887. HPLC: 96% (CH_3CN/H_2O = 1:1 (0.1% HCO_2H)).

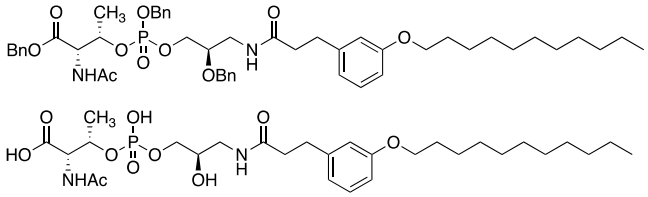
Synthesis of 10b (Figure 27). *Compound **84**.*



L-*allo*-Threonine (1190.2 mg, 9.9916 mmol) was suspended in AcOH (5 mL) and Ac_2O (1.1 mL). After stirring at room temperature for 24.5 h, the solvent was evaporated. The crude **83** and TBAI (1103.1 mg, 2.9946 mmol) were dissolved in anhydrous THF (30 mL) and Et_3N (5.0 mL). BnBr (1.75 mL) was added at 0 °C, and the mixture was stirred at room temperature under an Ar atmosphere for 18.1 h. H_2O (20 mL) was added, and the whole was extracted three times with AcOEt (30 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated. The residue was column-chromatographed on an open column with silica gel twice (first, *n*-hexane/acetone = 2:1 to 1:1, second, *n*-hexane/acetone = 3:2) to afford pure **84** (54.6 mg, 0.2172 mmol) as a colorless oil. As the isomerization of the α-amino group occurred to afford the mixture of **84** and **85** and the separation of diastereomers was difficult, the yield of pure compound was low (Figure 27).

^{1}H NMR (400 MHz, $CDCl_3$): 7.404−7.328 (5H, m), 6.488−6.474 (1H, m), 5.251−5.180 (2H, m), 4.730 (1H, dd, J = 7.0 Hz, 3.3 Hz), 4.233−4.160 (1H, m), 3.388 (1H, d, J = 6.4 Hz), 2.072 (3H, s), 1.114 (3H, d, J = 6.5 Hz).

*Compounds **86** and **10b**.*



Compounds **84** (42.1 mg, 0.168 mmol) and **4** (192.5 mg, 0.2670 mmol) were dissolved in CH_2Cl_2 and toluene. To remove traces of water, the solution was evaporated. The mixture was dissolved in anhydrous CH_2Cl_2 (0.75 mL). A solution of 1*H*-tetrazole (23.0 mg, 0.328 mmol) in anhydrous THF (0.75 mL) was added at 0 °C. The mixture was stirred at room temperature under an Ar atmosphere for 2.1 h. TBHP in decane (5.0−6.0 M) (61 μL) was added, and the mixture was stirred at room temperature for 1.5 h. H_2O (10 mL) was added, and the whole was extracted three times with CH_2Cl_2 (10 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated. The residue was column-chromatographed on a flash column with silica gel ($CHCl_3$/AcOEt = 2:1 to *n*-hexane/acetone = 3:2) to afford **86** containing **84** as a colorless oil (114.7 mg, 0.1293 mmol).

Crude **86** (113.5 mg, 0.1280 mmol) and Pd/C (25.5 mg) were dissolved in MeOH (5.0 mL). The mixture was stirred at room temperature under a H_2 atmosphere for 26.4 h. The mixture was filtered on Celite, and the solvent was evaporated. The residue was purified by reversed-phase medium-pressure liquid chromatography (CH_3CN/H_2O = 1:1, containing 0.1% HCOOH) to afford **10b** as a colorless powder (28.3 mg, 0.0459 mmol, 27% from **84**).

^{1}H NMR (400 MHz, DMSO-d_6): 12.774 (1H, br s), 8.355 (1H, d, J = 6.7 Hz), 7.884 (1H, t, J = 5.6 Hz), 7.149 (1H, dd, J = 8.1 Hz, 8.1 Hz), 6.754−6.699 (3H, m), 4.584−4.536 (1H, m), 4.487−4.456 (1H, m), 3.912 (2H, t, J = 6.5 Hz), 3.773−3.591 (3H, m), 3.180−3.119 (1H, m), 3.057−2.993 (1H, m), 2.757 (2H, t, J = 7.9 Hz), 2.375 (2H, t, J = 7.9 Hz), 1.882 (3H, s), 1.684 (2H, quin, J = 6.9 Hz), 1.409−1.253 (19H, m), 0.855 (3H, t, J = 6.8 Hz). HRMS (ESI-TOF, [M − H]$^-$): calcd for $C_{29}H_{48}N_2O_{10}P^-$, 615.3052; found, 615.3078. Anal. Calcd for $C_{29}H_{49}N_2O_{10}P{\cdot}0.5H_2O$: C, 55.67; H, 8.06; N, 4.48. Found: C, 55.53; H, 7.86; N, 4.65. HPLC: 97% (CH_3CN/H_2O = 1:1 (0.1% HCO_2H)).

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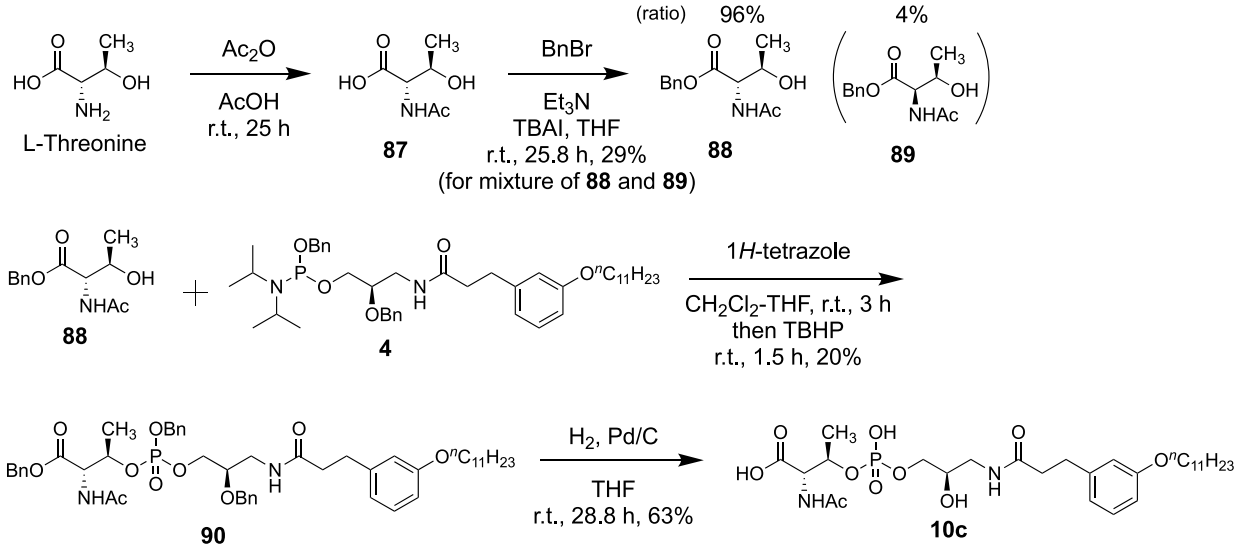
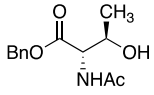


Figure 28. Synthesis of **10c**.

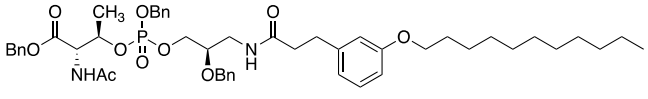
Synthesis of 10c (Figure 28). *Compound **88**.*



L-Threonine (1191.4 mg, 10.002 mmol) was suspended in AcOH (5 mL) and Ac_2O (1.1 mL). After stirring at room temperature for 25 h, the solvent was evaporated. The crude **87** and TBAI (1104.3 mg, 2.9978 mmol) were dissolved in anhydrous THF (30 mL) and Et_3N (4.0 mL). BnBr (1.4 mL) was added at 0 °C, and the mixture was stirred at room temperature under an Ar atmosphere for 25.8 h. H_2O (20 mL) was added, and the whole was extracted three times with AcOEt (30 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated. The residue was column-chromatographed on an open column with silica gel twice (first, *n*-hexane/acetone = 3:2 to 1:1) to afford **88** as a colorless solid (720.4 mg, 2.867 mmol, 29% from L-threonine). As the obtained **88** contained a small amount of **89**, the compound was used after recrystallization (Figure 28).

^{1}H NMR (400 MHz, $CDCl_3$): 7.398−7.320 (5H, m), 6.232 (1H, d, J = 8.6 Hz), 5.244−5.169 (2H, m), 4.654 (1H, dd, J = 8.9 Hz, 2.4 Hz), 4.393−4.327 (1H, m), 2.080 (3H, s), 1.982 (1H, d, J = 4.8 Hz), 1.212 (3H, d, J = 6.4 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): 170.94, 170.61, 135.20, 128.67, 128.53, 128.21, 68.12, 67.37, 57.22, 23.14, 20.00. HRMS (ESI-TOF, $[M + Na]^+$): calcd for $C_{13}H_{17}NNaO_4^+$, 274.1050; found, 274.1049. mp 102.5−103.3 °C (colorless needles, recrystallized from *n*-hexane/CH_2Cl_2).

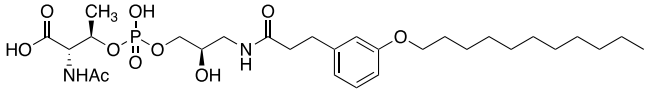
*Compound **90**.*



Compounds **88** (42.3 mg, 0.168 mmol) and **4** (191.2 mg, 0.2652 mmol) were dissolved in CH_2Cl_2 and toluene. To remove traces of water, the solution was evaporated. The mixture was dissolved in anhydrous CH_2Cl_2 (0.8 mL). A solution of 1*H*-tetrazole (22.9 mg, 0.327 mmol) in anhydrous THF (0.8 mL) was added at 0 °C. The mixture was stirred at room temperature under an Ar atmosphere for 3 h. TBHP in decane (5.0−6.0 M) (60 μL) was added, and the mixture was stirred at room temperature for 1.5 h. H_2O (10 mL) was added, and the whole was extracted three times with CH_2Cl_2 (10 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated. The residue was column-chromatographed on a flash column with silica gel twice (first, $CHCl_3$/AcOEt = 1:1 to *n*-hexane/acetone = 1:1, second, *n*-hexane/acetone = 2:1) to afford **90** as a colorless oil (30.1 mg, 0.0339 mmol, 20%).

^{1}H NMR (400 MHz, $CDCl_3$): (a mixture of diastereomers) 7.350−7.259 (15H, m), 7.173−7.127 (1H, m), 6.808−6.648 (4H, m), 5.937−5.879 (1H, m), 5.212−5.147 (1H, m), 5.083−4.919 (4H, m), 4.833−4.792 (1H, m), 4.592−4.563 (1H, m), 4.471−4.432 (1H, m), 3.966−3.834 (4H, m), 3.625−3.533 (1H, m), 3.457−3.314 (2H, m), 2.903−2.849 (2H, m), 2.492−2.346 (2H, m), 2.082 and 2.068 (3H, 2s), 1.785−1.711 (2H, m), 1.462−1.393 (2H, m), 1.365−1.254 (17H, m), 0.880 (3H, t, J = 6.9 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): (mixtures of diastereomers) 172.58, 172.34, 170.72, 170.70, 169.43, 169.35, 159.28, 142.28, 142.26, 137.63, 137.60, 135.53, 135.48, 134.97, 134.95, 129.41, 128.73, 128.68, 128.64, 128.62, 128.58, 128.57, 128.53, 128.46, 128.43, 128.39, 128.20, 128.03, 128.00, 127.93, 127.89, 127.88, 120.40, 114.68, 112.03, 75.87, 75.81, 75.68, 75.62, 75.52, 75.48, 75.44, 71.95, 71.87, 69.67, 69.64, 69.61, 69.58, 67.86, 67.68, 67.56, 66.52, 66.46, 66.03, 65.97, 56.38, 56.31, 56.25, 39.28, 39.05, 38.14, 31.87, 31.60, 29.58, 29.55, 29.39, 29.30, 29.24, 26.04, 22.91, 22.65, 18.49, 18.40, 14.09. HRMS (ESI-TOF, $[M + NH_4]^+$): calcd for $C_{50}H_{71}N_3O_{10}P^+$, 904.4872; found, 904.4855.

*Compound **10c**.*



Compound **90** (29.9 mg, 0.0337 mmol) and Pd/C (4.8 mg) were dissolved in THF (3.0 mL). The mixture was stirred at room temperature under a H_2 atmosphere for 24.3 h. Pd/C (7.4 mg) suspended in THF (1.0 mL) was added, and the mixture was stirred at room temperature under a H_2 atmosphere for 4.5 h. The mixture was filtered on Celite, and the solvent was evaporated. The residue was purified by reversed-phase medium-pressure liquid chromatography (CH_3CN/H_2O = 4:6 to 7:3, containing 0.1% HCOOH) to afford **10c** as a colorless powder (13.0 mg, 0.0211 mmol, 63%).

^{1}H NMR (400 MHz, DMSO-d_6): 8.329 (1H, br s), 7.906 (1H, t, J = 5.5 Hz), 7.148 (1H, dd, J = 8.1 Hz, 8.1 Hz), 6.754−6.699 (3H, m), 4.543 (1H, br s), 4.421−4.391 (1H, m), 3.911 (2H, t, J = 6.5 Hz),

https://doi.org/10.1021/acs.jmedchem.1c00347
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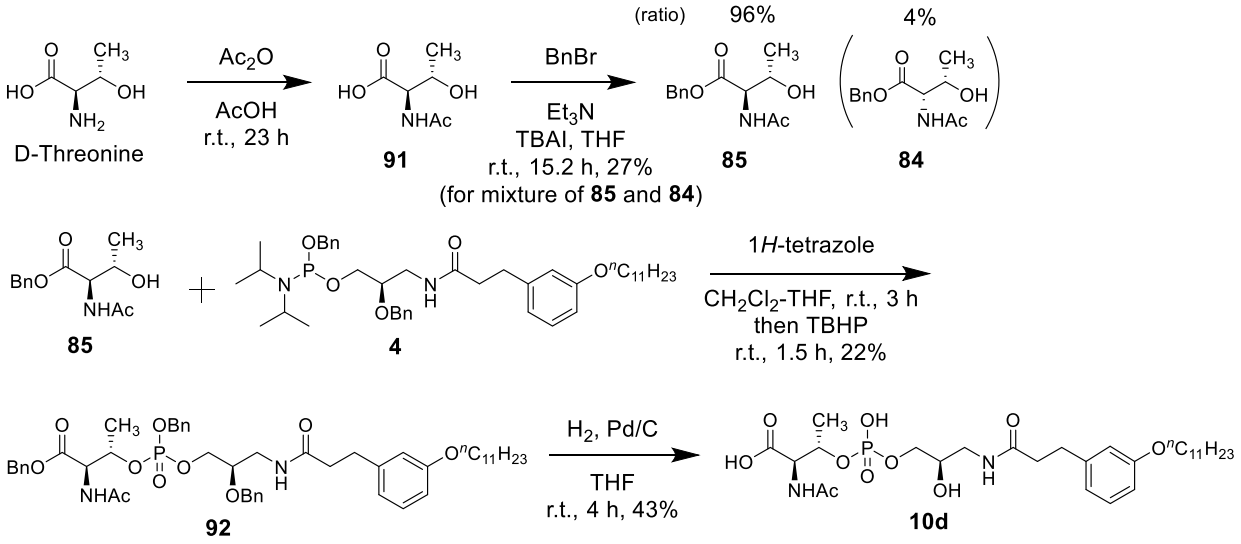
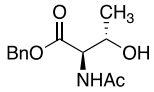


Figure 29. Synthesis of **10d**.

3.704−3.673 (2H, m), 3.636−3.581 (1H, m), 3.178−3.116 (1H, m), 3.050−2.986 (1H, m), 2.758 (2H, t, J = 7.9 Hz), 2.376 (2H, t, J = 7.9 Hz), 1.891 (3H, s), 1.684 (2H, quin, J = 6.9 Hz), 1.393−1.185 (19H, m), 0.855 (3H, t, J = 6.8 Hz). HRMS (ESI-TOF, $[M - H]^-$): calcd for $C_{29}H_{48}N_2O_{10}P^-$, 615.3052; found, 615.3081. HPLC: 97% (CH_3CN/H_2O = 1:1 (0.1% HCO_2H)).

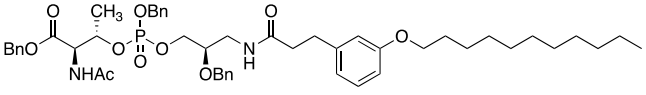
Synthesis of 10d (Figure 29). *Compound **85**.*



D-Threonine (1192.1 mg, 10.008 mmol) was suspended in AcOH (5 mL) and Ac_2O (1.1 mL). After stirring at room temperature for 23 h, the solvent was evaporated. The crude **91** and TBAI (1105.5 mg, 3.0011 mmol) were dissolved in anhydrous THF (30 mL) and Et_3N (4.0 mL). BnBr (1.4 mL) was added at 0 °C, and the mixture was stirred at room temperature under an Ar atmosphere for 15.2 h. H_2O (30 mL) was added, and the whole was extracted three times with AcOEt (30 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated. The residue was column-chromatographed on an open column with silica gel twice (first, *n*-hexane/acetone = 1:1, 2:3 to 1:2) to afford **85** as a colorless solid (671.9 mg, 2.674 mmol, 27% from D-threonine). As the obtained **85** contained a small amount of **84**, the compound was used after recrystallization (Figure 29).

^{1}H NMR (400 MHz, $CDCl_3$): 7.394−7.317 (5H, m), 6.310 (1H, d, J = 8.5 Hz), 5.237−5.164 (2H, s), 4.648 (1H, dd, J = 8.9 Hz, 2.4 Hz), 4.389−4.323 (1H, m), 2.184 (1H, d, J = 4.9 Hz), 2.074 (3H, s), 1.206 (3H, d, J = 6.4 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): 170.95, 170.70, 135.19, 128.65, 128.51, 128.19, 68.07, 67.35, 57.26, 23.10, 19.99. HRMS (ESI-TOF, $[M + H]^+$): calcd for $C_{13}H_{18}NO_4^+$, 252.1230; found, 252.1217.

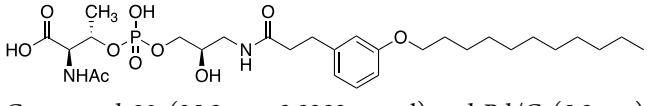
*Compound **92**.*



Compounds **85** (34.4 mg, 0.137 mmol) and **4** (157.8 mg, 0.2189 mmol) were dissolved in CH_2Cl_2 and toluene. To remove traces of water, the solution was evaporated. The mixture was dissolved in anhydrous CH_2Cl_2 (0.7 mL). A solution of 1*H*-tetrazole (19.9 mg, 0.284 mmol) in anhydrous THF (0.7 mL) was added at 0 °C. The mixture was stirred at room temperature under an Ar atmosphere for 3 h. TBHP in decane (5.0−6.0 M) (50 μL) was added, and the mixture was stirred at room temperature for 1.5 h. H_2O (10 mL) was added, and the whole was extracted three times with CH_2Cl_2 (10 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated. The residue was column-chromatographed on a flash column with silica gel twice (first, $CHCl_3$/AcOEt = 1:1 to *n*-hexane/acetone = 1:1, second, *n*-hexane/acetone = 2:1) to afford **92** as a colorless oil (26.5 mg, 0.0299 mmol, 22%).

^{1}H NMR (400 MHz, $CDCl_3$): (mixtures of diastereomers) 7.348−7.259 (15H, m), 7.180−7.126 (1H, m), 6.764−6.702, (3.6H, m), 6.622 (0.4H, d, J = 9.1 Hz), 5.969 and 5.828 (1H, 2t, J = 5.9 Hz), 5.214−5.145 (1H, m), 5.083−4.944 (4H, m), 4.830−4.764 (1H, m), 4.600−4.557 (1H, m), 4.489−4.427 (1H, m), 3.985−3.833 (4H, m), 3.634−3.279 (3H, m), 2.875 (2H, t, J = 7.8 Hz), 2.420−2.365 (2H, m), 2.071 and 2.049 (3H, 2s), 1.787−1.711 (2H, m), 1.464−1.395 (2H, m), 1.356−1.255 (17H, m), 0.880 (3H, t, J = 6.9 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): (mixtures of diastereomers) 172.39, 172.25, 170.76, 170.70, 169.56, 169.40, 159.29, 142.28, 142.24, 137.65, 137.61, 135.57, 135.50, 135.43, 134.97, 129.41, 128.76, 128.67, 128.62, 128.58, 128.55, 128.53, 128.45, 128.38, 128.26, 128.06, 128.03, 127.94, 127.89, 120.39, 114.68, 114.65, 112.08, 112.02, 75.80, 75.74, 75.69, 75.59, 75.53, 75.47, 72.00, 71.97, 69.72, 69.66, 69.61, 69.56, 67.86, 67.71, 67.58, 66.55, 66.49, 56.44, 56.35, 56.28, 39.39, 39.31, 38.15, 38.13, 31.88, 31.60, 29.59, 29.56, 29.40, 29.30, 26.05, 22.91, 22.90, 22.66, 18.52, 18.47, 14.09. HRMS (ESI-TOF, $[M + NH_4]^+$): calcd for $C_{50}H_{71}N_3O_{10}P^+$, 904.4872; found, 904.4846.

*Compound **10d**.*



Compound **92** (25.9 mg, 0.0292 mmol) and Pd/C (5.9 mg) were dissolved in THF (3.0 mL). The mixture was stirred at room temperature under a H_2 atmosphere for 4 h. The mixture was filtered on Celite, and the solvent was evaporated. The residue was purified by reversed-phase medium-pressure liquid chromatography (CH_3CN/H_2O = 3:7 to 7:3, containing 0.1% HCOOH) to afford **10d** as a colorless powder (7.8 mg, 0.0126 mmol, 43%).

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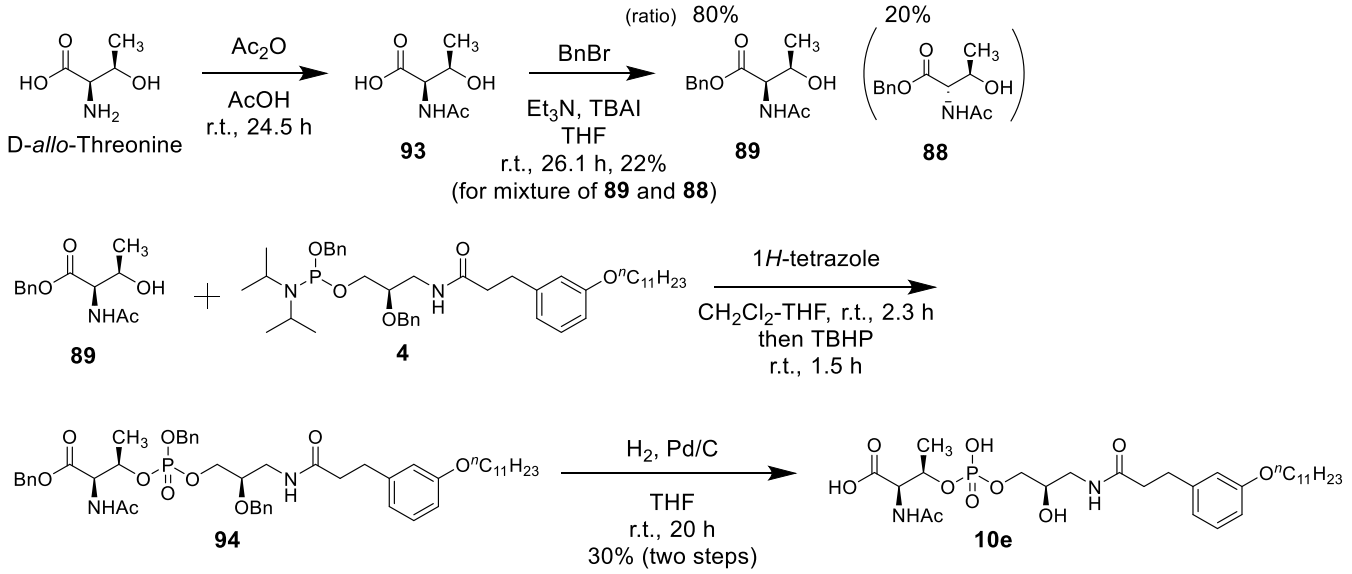
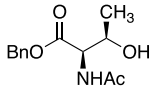


Figure 30. Synthesis of **10e**.

^{1}H NMR (400 MHz, DMSO-d_6): 8.298 (1H, d, J = 7.0 Hz), 7.899 (1H, t, J = 5.6 Hz), 7.145 (1H, dd, J = 8.1 Hz, 8.1 Hz), 6.751−6.696 (3H, m), 4.557 (1H, br s), 4.434−4.406 (1H, m), 3.908 (2H, t, J = 6.4 Hz), 3.714−3.592 (3H, m), 3.201−3.141 (1H, m), 3.031−2.968 (1H, m), 2.756 (2H, t, J = 7.9 Hz), 2.375 (2H, t, J = 7.9 Hz), 1.891 (3H, s), 1.680 (2H, quin, J = 6.8 Hz), 1.390−1.188 (19H, m), 0.851 (3H, t, J = 6.8 Hz). HRMS (ESI-TOF, $[M - H]^-$): calcd for $C_{29}H_{48}N_2O_{10}P^-$, 615.3052; found, 615.3057. HPLC: >99% (CH_3CN/H_2O = 1:1 (0.1% HCO_2H)).

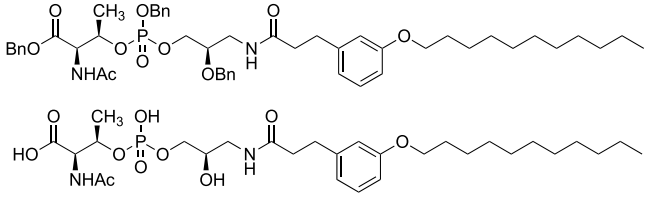
Synthesis of 10e (Figure 30). *Compound **89**.*



D-*allo*-Threonine (1191.0 mg, 9.9983 mmol) was suspended in AcOH (5 mL) and Ac_2O (1.1 mL). After stirring at room temperature for 24.5 h, the solvent was evaporated. The crude **93** and TBAI (1103.0 mg, 2.9943 mmol) were dissolved in anhydrous THF (30 mL) and Et_3N (4.0 mL). BnBr (1.4 mL) was added at 0 °C, and the mixture was stirred at room temperature under an Ar atmosphere for 26.1 h. H_2O (40 mL) was added, and the whole was extracted three times with AcOEt (40 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated. The residue was column-chromatographed on an open column with silica gel (*n*-hexane/acetone = 3:2) to afford pure **89** as a colorless oil (50.7 mg, 0.202 mmol). As the isomerization of the α-amino group occurred to afford the mixture of **89** and **88** and the separation of diastereomers was difficult, the yield of pure compound was low (Figure 30).

^{1}H NMR (400 MHz, $CDCl_3$): 7.392−7.323 (5H, m), 6.524 (1H, d, J = 6.1 Hz), 5.230 (1H, d, J = 12.2 Hz), 5.189 (1H, d, J = 12.2 Hz), 4.723 (1H, dd, J = 7.1 Hz, 3.3 Hz), 4.189 (1H, qd, J = 6.5 Hz, 3.4 Hz), 2.065 (3H, s), 1.117 (3H, d, J = 6.5 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): 171.21, 170.02, 134.82, 128.64, 128.61, 128.32, 69.08, 67.56, 58.38, 22.99, 18.58. HRMS (ESI-TOF, $[M + Na]^+$): calcd for $C_{13}H_{17}NNaO_4^+$, 274.1050; found, 274.1064.

*Compounds **94** and **10e**.*



Compounds **89** (44.7 mg, 0.178 mmol) and **4** (182.5 mg, 0.2531 mmol) were dissolved in CH_2Cl_2 and toluene. To remove traces of water, the solution was evaporated. The mixture was dissolved in anhydrous CH_2Cl_2 (0.75 mL). A solution of 1*H*-tetrazole (25.8 mg, 0.368 mmol) in anhydrous THF (0.75 mL) was added at 0 °C. The mixture was stirred at room temperature under an Ar atmosphere for 2.3 h. TBHP in decane (5.0−6.0 M) (65 μL) was added, and the mixture was stirred at room temperature for 1.5 h. H_2O (10 mL) was added, and the whole was extracted three times with CH_2Cl_2 (10 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The solvent was evaporated. The residue was column-chromatographed on a flash column with silica gel ($CHCl_3$/AcOEt = 2:1 to *n*-hexane/acetone = 1:1) to afford **94** containing **89** (6%, calculated from CH_3 peak of ^{1}H NMR) as a colorless oil (99.0 mg).

Crude **94** (98.0 mg) and Pd/C (15.2 mg) were dissolved in THF (5.0 mL). The mixture was stirred at room temperature under a H_2 atmosphere for 20 h. The mixture was filtered on Celite, and the solvent was evaporated. The residue was purified by reversed-phase medium-pressure liquid chromatography (CH_3CN/H_2O = 35:65 to 70:30, containing 0.1% HCOOH) to afford **10e** as a colorless powder (33.3 mg, 0.0540 mmol, 30% from **89**).

^{1}H NMR (400 MHz, DMSO-d_6): 8.308 (1H, d, J = 7.6 Hz), 7.879 (1H, t, J = 5.7 Hz), 7.147 (1H, dd, J = 8.1 Hz, 8.1 Hz), 6.752−6.697 (3H, m), 4.593−4.544 (1H, m), 4.512−4.480 (1H, m), 3.909 (2H, t, J = 6.5 Hz), 3.756−3.695 (2H, m), 3.669−3.596 (1H, m), 3.182−3.120 (1H, m), 3.048−2.984 (1H, m), 2.755 (2H, t, J = 7.9 Hz), 2.374 (2H, t, J = 7.9 Hz), 1.882 (3H, s), 1.681 (2H, quin, J = 6.9 Hz), 1.405−1.355 (2H, m), 1.268−1.251 (17H, m), 0.852 (3H, t, J = 6.9 Hz). HRMS (ESI-TOF, $[M - H]^-$): calcd for $C_{29}H_{48}N_2O_{10}P^-$, 615.3052; found, 615.3031. Anal. Calcd for $C_{29}H_{49}N_2O_{10}P{\cdot}0.5H_2O$: C, 55.67; H, 8.06; N, 4.48. Found: C, 55.45; H, 7.76; N, 4.54. HPLC: 99% (CH_3CN/H_2O = 1:1 (0.1% HCO_2H)).

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Homology Modeling of Human GPR174. Human GPR174 homology models were built using MODELLER 9.20[24] based on the crystal structure of zebrafish LPA_6 (PDBID: 5XSZ)[21] and the human GPR174 sequence obtained from UniProt (code Q9BXC1).[25] Only part of the GPR174 sequence (from Thr15 to Ser311) for which the template structure was available was used for modeling. Sequence alignment was conducted with ClustalW[26] using a Multiple Sequence Viewer (Schrödinger, LLC, NY) as a graphical interface. The alignment was manually modified to fill the gap in TM5. The final alignment is shown in Figure S5. Ten models were constructed and the model that had the best MODELLER discrete optimized protein energy (DOPE) score was used in the next docking process.

Induced Fit Docking of GPR174 Antagonist 7d and MD-Based Minimization. The constructed GPR174 homology model was refined with Protein Preparation Wizard in Maestro (Schrödinger 2017-4, Schrödinger, LLC, NY), capping the protein termini and using PROPKA[27] with pH 7.0 in the H-bond assignment step. Suitable 3D coordinates and states of compound **7d** were prepared using the OPLS3 force field and the Epik calculation with pH 7.0 on LigPrep (version 44011, Schrödinger 2017-4, Schrödinger, LLC, NY). Both the carboxyl group and the phosphodiester were ionized to afford the total molecular charge −2.

Compound **7d** was docked to the GPR174 homology model using the standard Induced Fit Docking (IFD) protocol (Schrödinger 2017-4, Schrödinger, LLC, NY).[28] Assuming the binding pocket of GPR174 is similar to that of GPR34, the box center was defined as the centroid of the bound compound in the GPR34 homology model[20] superimposed on the GPR174 homology model.

The obtained models (models 1 and 2) of the GPR174−compound **7d** complex were subjected to 100 ns MD-based energy minimization using Desmond (Schrödinger 2017-4, Schrödinger, LLC, NY). Initial ligand−receptor complex models were aligned to the template LPA_6 structure obtained from the Orientations of Proteins in Membranes (OPM) database[29] and embedded in 1-palmitoyl-2-oleoyl-phosphatidylethanolamine (POPE) membrane, referring to the membrane position in the OPM database. Na^+ ion extracted from the protease-activated receptor 1 (PAR1) crystal structure (PDBID: 3VW7)[30] was placed in the Na^+ binding pocket. The system was placed in TIP3P water molecules solvated with 0.15 M NaCl. The OPLS3 force field was used. Production MD was performed in the NPγT ensemble at 300 K, 1.01325 bar and 4000 bar·Å as surface tension using Langevin dynamics. Long-range electrostatic interactions were computed using the Smooth Particle Mesh Ewald method.

The figures were created on the graphical user interface of Maestro (Schrödinger, LLC, NY).

TGFα Shedding Assay. Both agonistic and antagonistic activities were evaluated by the TGFα shedding assay. The TGFα shedding assay was performed as described previously[20] with several minor modifications as follows. HEK293FT cells were used instead of HEK293A cells. After 24 h of transfection of AP-TGFα and mouse GPR174-expressing vectors, the cells were detached, suspended in Hank's balanced salt solution, seeded into 96-well plates, and stimulated with varying concentrations of compounds. After 90 min of incubation, the plates were centrifuged, and the supernatants were transferred into other plates. Then, *p*-NPP (1 M) was added to both plates and OD405 was measured before and after 30 min of incubation by a SpectraMax Plus 384 Microplate Reader (Molecular Devices). The agonistic activities of each compound were calculated by subtracting background responses in the mock-transfected cells (GPR174 (−)) from the responses in the GPR174-transfected cells. For evaluation of antagonistic activities, each compound was added simultaneously with a GPR174 agonist (**LysoPalloT-NH-C3-ph-*m*-O-C11**,[18] final concentration: 100 nM). The amount of released AP-TGFα was calculated similarly to the evaluation of agonistic activities. AP-TGFα release and GPCR activation were calculated as follows.

$$\text{AP-TGF}\alpha\text{ release (\%)} = \Delta\text{OD405Sup}/(\Delta\text{OD405Sup} + \Delta\text{OD405Cell}) \times 100 \times 1.25$$

Multiplication by 1.25 means the conversion of AP-TGFα release in actually transferred supernatant (80 μL) to AP-TGFα release in total supernatant (100 μL)

$$\text{GPCR activation (\%)} = \text{AP-TGF}\alpha\text{ release under stimulated condition (\%)} - \text{AP-TGF}\alpha\text{ release under non-stimulated condition (\%)}$$

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.1c00347.

Full biological data, HPLC charts of lead compounds, and alignment of amino acid sequences for homology modeling (PDF)

PDB file of docking model (model 1 and model 2) (PDB) (PDB)

Molecular formula strings (CSV)

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Author Contributions

M.S. and A.U. have equal contribution. M.S., M.I., L.C., G.Y., Y.O., and T.O. (The University of Tokyo) performed the chemical studies including design and synthesis of compounds and simulations. M.S. (The University of Tokyo), A.U., A.I., and J.A. (Tohoku University and The University of Tokyo) performed biological studies including the TGFα shedding assay. M.S. (design and synthesis of molecules and simulations) and A.U. (biological assay) contributed equally to this work.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

AP-TGFα, alkaline phosphatase-tagged transforming growth factor-α; CSA, camphorsulfonic acid; DIPEA, *N*,*N*-diisopropylethylamine; DOPE, discrete optimized protein energy; EDCI, 1-(3-(dimethylamino)-propyl)-3-ethylcarbodiimide; HOBT, 1-hydroxybenzotriazole; IFD, induced fit docking; IL-2, interleukin-2; KO, knock-out; LPA, lysophosphatidic acid; LPA_6, lysophosphatidic acid receptor 6; LPL, lysophospholipids; LysoPS, lysophosphatidylserine; MMTr, monomethoxytrityl; *p*-NPP, *para*-nitrophenyl phosphate; NPγT, constant normal pressure and lateral surface tension of membranes and constant temperature; OPLS, optimized potentials for liquid simulations; OPM, Orientations of Proteins in Membranes; PAR1, protease-activated receptor 1; POPE, 1-palmitoyl-2-oleoyl-phosphatidylethanolamine; PS, phosphatidylserine; SEM, standard error of the mean; TBAI, tetrabutylammonium iodide; TGFα, transforming growth factor-α; TM, transmembrane helix; Treg, regulatory T cell

■ ADDITIONAL NOTE

[a]The number of peaks does not seem enough in the ^{13}C NMR spectrum, but it agreed with reported data[19]

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