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Studies on Scavenger Receptor Inhibitors. Part 1: Synthesis and Structure–Activity Relationships of Novel Derivatives of Sulfatides

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Abstract—Scavenger receptors have been proven to be implicated in the formation of atherosclerotic lesions. A series of novel derivatives of sulfatides were synthesized, and their inhibitory activities against incorporation of DiI-acetyl-LDL into macrophages were evaluated in order to clarify the structure–activity relationships of sulfatides as a scavenger receptor inhibitor and find out novel inhibitors with synthetic easiness. The chemical modification of the substructures of sulfatides led to the establishment of the following structure–activity relationships; (1) the ceramide moiety can be replaced with another structure bearing two long chains, (2) the galactose moiety can be replaced with another structure or be deleted without a large decrease in the inhibitory activity, (3) the sulfate moiety was crucial, and it was the most preferable functional group for a potent inhibitory activity. The inhibitory activity of (*S*)-2-octadecanoylamino-2-tetradecylcarbamoyl)ethyl sulfate sodium salt (**3a**) against incorporation of DiI-acetyl-LDL into macrophages was proven to be based on the inhibition against the binding of acetyl-LDL to the surface of macrophages. We discovered novel scavenger receptor inhibitors with synthetic easiness, such as (*S*)-2-octadecanoylamino-2-(tetradecylcarbamoyl)ethyl sulfate sodium salt (**3a**) and 2-octadecanoylamino-1-(octadecanoylaminomethyl)ethyl sulfate sodium salt (**13q**).

Introduction

Atherosclerosis in early stage is characterized by the presence of foam cells in the arterial intima. Foam cells, which are filled with cholesterol ester-rich lipid droplets, lead to atherosclerotic plaques. It is generally accepted that the formation of the foam cells begins with the attachment of circulating monocytes to the lumenal surface of endothelial cells. Then, the monocytes migrate into the subendothelial space, where they differentiate into macrophages. The resulting macrophages uptake lipoproteins, and accumulate a large amount of cholesterol ester derived from the lipoproteins, which results in the formation of the foam cells.[1–4]

Various evidences have demonstrated that modified low density lipoproteins (modified LDLs), not native low density lipoprotein, are responsible for the accumulation of cholesterol ester in the macrophages. Modified LDLs are recognized by special molecules, scavenger receptors, expressed in the surface of the macrophages. After the binding of modified LDLs to scavenger receptors, the formed complexes are delivered to lysosomes by endocytosis. In lysosomes, modified LDLs are degradated to their protein portion and cholesterol ester portion, and the latter is further hydrolyzed to free cholesterol by a lysosomal cholesterol esterase. Free cholesterol generated in lysosomes is then transported into cytoplasm, where it is re-esterified to cholesterol ester by acyl-coenzyme A/cholesterol acyltransferase (ACAT), leading to cholesterol ester-rich lipid droplets (Fig. 1).

The presence of scavenger receptors which can incorporate acetyl-LDL, one of modified LDLs, was predicted by Brown and Goldstein 23 years ago.[5] Then, Kodama et al. succeeded in the cloning of complementary DNA of scavenger receptor for acetyl-LDL in 1990, which led to the clarification of the substructures of the scavenger receptor.[6,7] After that, novel scavenger receptors which can recognize modified LDLs have been identified by several research groups,[8–15] and

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now the scavenger receptors cloned by Kodama et al. are called class A scavenger receptor.

If the binding of modified LDLs to scavenger receptors is blocked, it could be thought that the accumulation of cholesterol ester and the subsequent formation of foam cells do not happen, because modified LDLs are not incorporated into the macrophages. Therefore, compounds with an inhibitory activity against the binding of modified LDLs to scavenger receptors (referred to as scavenger receptor inhibitor) should be effective in the treatment of atherosclerosis. Recently, Suzuki et al. reported that class A scavenger receptor/apolipoprotein E double-knockout mice developed 60% smaller atherosclerotic lesions than single-apolipoprotein E knockout littermates.[16] This was the first in vivo evidence that class A scavenger receptors are implicated in formation of atherosclerotic lesions, meaning that class A scavenger receptor inhibitors would prevent the progress of atherosclerosis.

Before the cloning of the scavenger receptors by Kodama et al., Brown and Goldstein had already found that negatively charged macromolecules, such as poly vinyl sulfate, polyG, and maleyl-LDL, inhibited the binding of ^{125}I-acetyl-LDL to the surface of macrophages.[17] Moreover, they discovered that sulfatides, a mixture of negatively charged glycolipids having two long chains (Fig. 2), also exhibited the inhibitory activity.[17] Therefore, sulfatides can be one of scavenger receptor inhibitors with low molecular weight.[18] However, we considered that the use of sulfatides itself for the treatment of atherosclerosis would be impractical, because sulfatides is a mixture of structurally related compounds. Chemical synthesis enables us to obtain a certain component of sulfatides, but there have been some problems in the chemical synthesis of sulfatides. One of them was the difficulty of the synthesis of the ceramide moiety which has two asymmetric carbons and one olefin group. Although many synthetic routes for ceramide have been reported,[19–21] all of them still have drawbacks such as many steps, low yield, and/or formation of isomer.

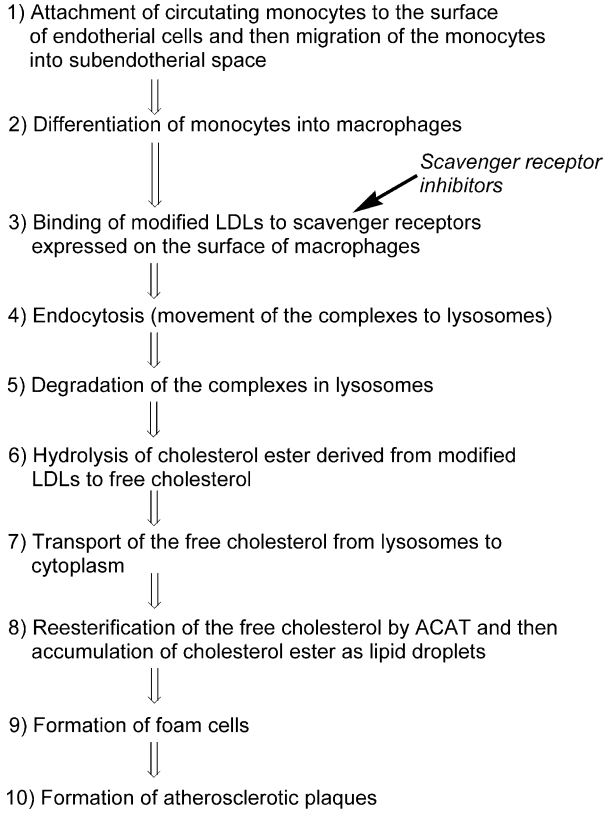


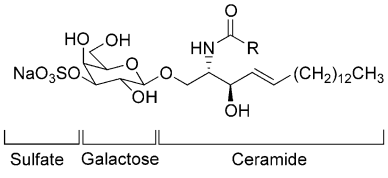
Figure 1. Schematic progress of atherosclerosis.

By the way, the structure–activity relationships (SARs) of sulfatides as scavenger receptor inhibitor have not been investigated yet, so it was unclear which substructures of sulfatides are important for its inhibitory activity. Herein, at first, we report the synthesis of novel derivatives of sulfatides. In addition, we describe the SARs of sulfatides as a scavenger receptor inhibitor.

Chemistry

The synthetic route for the peptide derivative **1a** was outlined in Scheme 1. 2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl bromide **15** was treated with *N*-(*tert*-butoxycarbonyl)-L-serine benzyl ester **16** in the presence of silver carbonate, silver perchlorate, and molecular sieves to give β-glycoside **17**. Removal of *tert*-butoxycarbonyl (Boc) group of compound **17** with trifluoroacetic acid (TFA) afforded amine **18**, which was acylated with octadecanoyl chloride. The obtained *N*-octadecanoyl serine benzyl ester **19** was hydrogenated in the presence of palladium–carbon (Pd–C), followed by condensation with tetradecylamine by use of 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDCI) and 1-hydroxybenzotriazole hydrate (HOBt) to yield peptide **21**. Removal of the four acetyl groups of compound **21** was achieved with sodium methoxide in methanol. According to the procedure reported by Guilbert et al.,[22] the obtained tetraol **22** was treated with di-*n*-butyltin oxide, followed by sulfation with sulfur trioxide-trimethylamine complex to afford the target compound **1a**, regioselectively.

Scheme 2 shows the synthetic route for the galactose-deleted derivatives of compound **1a**. To begin with, condensation of *N*-Boc-*O*-benzyl-L-serine **23** with alkylamine R^2NH_2 gave *N*-Boc-serinamides **24**. The Boc group of compounds **24** was removed by use of TFA to give amines **25**. Acylation of **25** with acyl chloride R^1COCl to yield *N*-acyl-*O*-benzyl-serinamides **26**. Hydrogenolysis of compounds **26** over Pd/C afforded



R = -$(CH_2)_{14}CH_3$, -$(CH_2)_{16}CH_3$, -$(CH_2)_{22}CH_3$, -$CHOH(CH_2)_{21}CH_3$, -$(CH_2)_{13}CH{=}CH(CH_2)_7CH_3$, -$CHOH(CH_2)_{12}CH{=}CH(CH_2)_7CH_3$, etc.

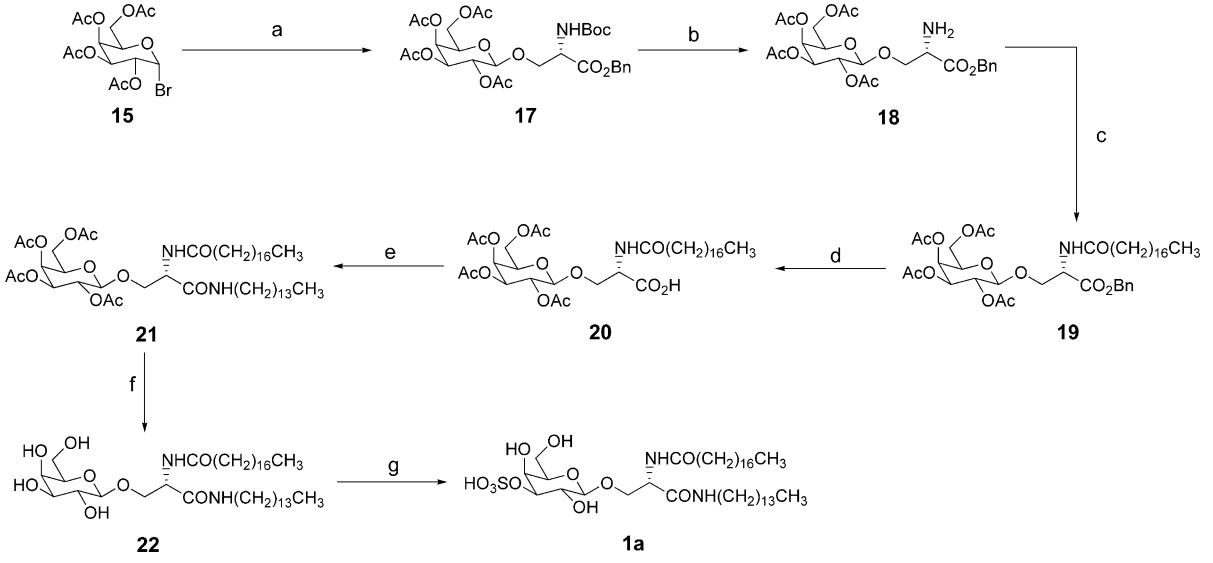
Figure 2. Structure of sulfatides.

alcohols **8**. Finally, compounds **8** were sulfated with chlorosulfonic acid in pyridine, followed by treatment with sodium carbonate to give the target compounds **3a** and **3c–j**. The enantiomer of **3a**, compound **3b**, was prepared from *N*-Boc-*O*-benzyl-D-serine in the same manner as compound **3a** (scheme not shown).

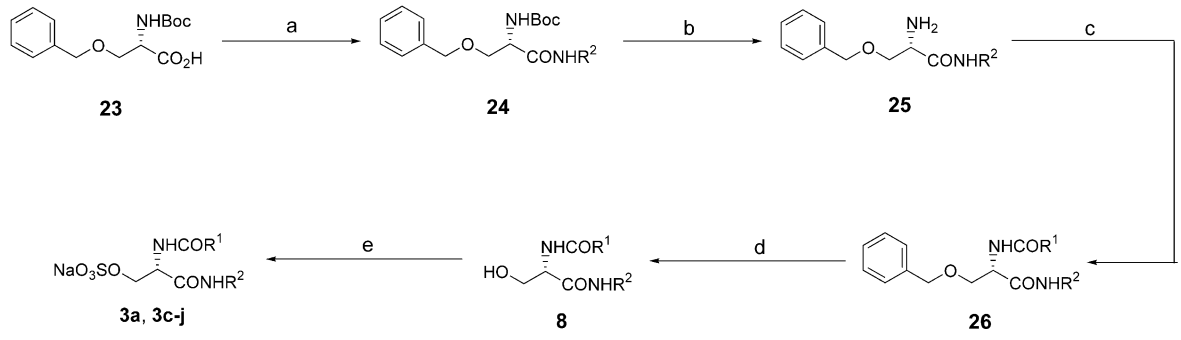
Three peptides **10a**, **27a**, and **28a** were prepared from the corresponding *N*-Boc-amino acids (**10n**, **27n**, and **28n**) in the same manner as compound **26**, as shown in Scheme 3. Then, *O*-benzyl-*N*-octadecanoyltyrosinamide **28a** was hydrogenated, and then sulfated in a harder condition to afford compound **2a** (Scheme 4). The benzyl ester of compound **27a** was reduced with sodium borohydride in methanol–tetrahydrofuran, followed by sulfation of the resulting alcohol **29a** to afford butyl sulfate **4a**. Removal of the benzyl group of compound **27a** by hydrogenolysis over Pd–C gave carboxylic acid **6a**. Sulfoxide **10a** was oxidized with 3-chloroperoxybenzoic acid (*m*-CPBA) to give sulfone **11a**.

Scheme 5 shows the synthetic route for thiol **9a** and ethylthiopropyl sulfate **5a**. At first, *N*-Boc-L-cystine **30n** was converted to cystinamide **30a** in three steps. The disulfide bond of **30a** was cleaved reductively with tributylphosphine to yield *N*-octadecanoylcysteinamide **9a**. Treatment of **9a** with 3-bromopropanol in the presence of Na_2CO_3, followed by sulfation of the resulting alcohol **31a** to afford 3-ethylthiopropyl sulfate **5a**.

Compounds **12j** and **13q–s** were synthesized according to the routes shown in Scheme 6. 2-Aminoethanol **32** or

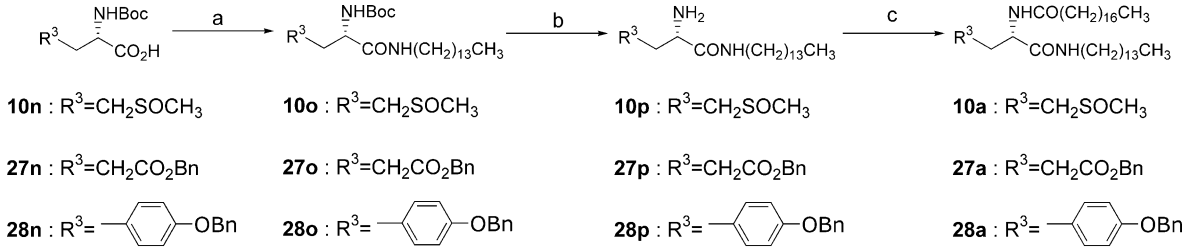


Scheme 1. Reagents: (a) *N*-(*tert*-butoxycarbonyl)-L-serine benzyl ester (**16**), Ag_2CO_3, $AgClO_4$, molecular sieves; (b) TFA; (c) $ClCO(CH_2)_{16}CH_3$, Et_3N; (d) Pd/C (10%), H_2; (e) $H_2N(CH_2)_{13}CH_3$, EDCI, HOBt; (f) NaOMe; (g) (i) *n*-Bu_2SnO; (ii) SO_3–Et_3N.

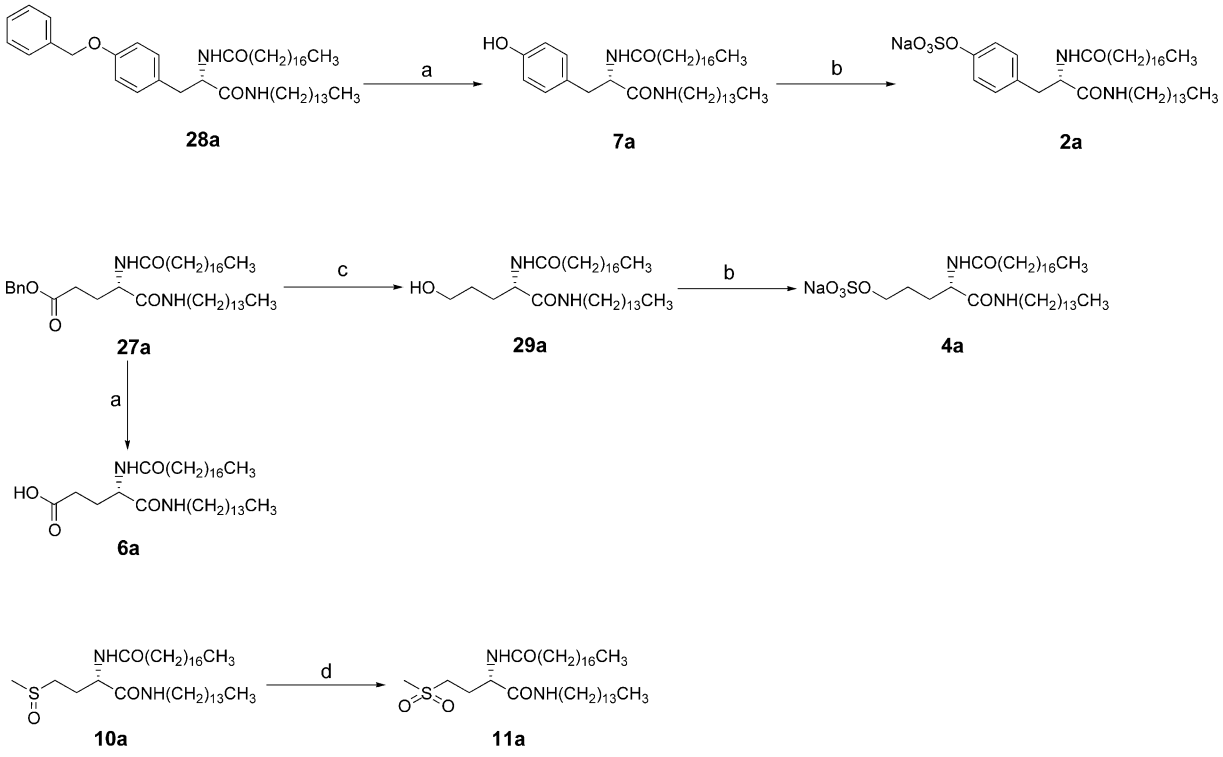


3a : $R^1=(CH_2)_{16}CH_3$, $R^2=(CH_2)_{13}CH_3$	**8a** : $R^1=(CH_2)_{16}CH_3$, $R^2=(CH_2)_{13}CH_3$	**8a** : $R^1=(CH_2)_{16}CH_3$, $R^2=(CH_2)_{13}CH_3$
3c : $R^1=CH_3$, $R^2=(CH_2)_{13}CH_3$	**8c** : $R^1=CH_3$, $R^2=(CH_2)_{13}CH_3$	**8c** : $R^1=CH_3$, $R^2=(CH_2)_{13}CH_3$
3d : $R^1=(CH_2)_8CH_3$, $R^2=(CH_2)_{13}CH_3$	**8d** : $R^1=(CH_2)_8CH_3$, $R^2=(CH_2)_{13}CH_3$	**8d** : $R^1=(CH_2)_8CH_3$, $R^2=(CH_2)_{13}CH_3$
3e : $R^1=(CH_2)_{20}CH_3$, $R^2=(CH_2)_{13}CH_3$	**8e** : $R^1=(CH_2)_{20}CH_3$, $R^2=(CH_2)_{13}CH_3$	**8e** : $R^1=(CH_2)_{20}CH_3$, $R^2=(CH_2)_{13}CH_3$
3f : $R^1=(CH_2)_{24}CH_3$, $R^2=(CH_2)_{13}CH_3$	**8f** : $R^1=(CH_2)_{24}CH_3$, $R^2=(CH_2)_{13}CH_3$	**8f** : $R^1=(CH_2)_{24}CH_3$, $R^2=(CH_2)_{13}CH_3$
3g : $R^1=(CH_2)_{16}CH_3$, $R^2=(CH_2)_2CH_3$	**8g** : $R^1=(CH_2)_{16}CH_3$, $R^2=(CH_2)_2CH_3$	**8g** : $R^1=(CH_2)_{16}CH_3$, $R^2=(CH_2)_2CH_3$
3h : $R^1=(CH_2)_{16}CH_3$, $R^2=(CH_2)_7CH_3$	**8h** : $R^1=(CH_2)_{16}CH_3$, $R^2=(CH_2)_7CH_3$	**8h** : $R^1=(CH_2)_{16}CH_3$, $R^2=(CH_2)_7CH_3$
3i : $R^1=(CH_2)_{16}CH_3$, $R^2=(CH_2)_{18}CH_3$	**8i** : $R^1=(CH_2)_{16}CH_3$, $R^2=(CH_2)_{18}CH_3$	**8i** : $R^1=(CH_2)_{16}CH_3$, $R^2=(CH_2)_{18}CH_3$
3j : $R^1=CH[(CH_2)_{13}CH_3]_2$, $R^2=(CH_2)_2CH_3$	**8j** : $R^1=CH[(CH_2)_{13}CH_3]_2$, $R^2=(CH_2)_2CH_3$	**8j** : $R^1=CH[(CH_2)_{13}CH_3]_2$, $R^2=(CH_2)_2CH_3$

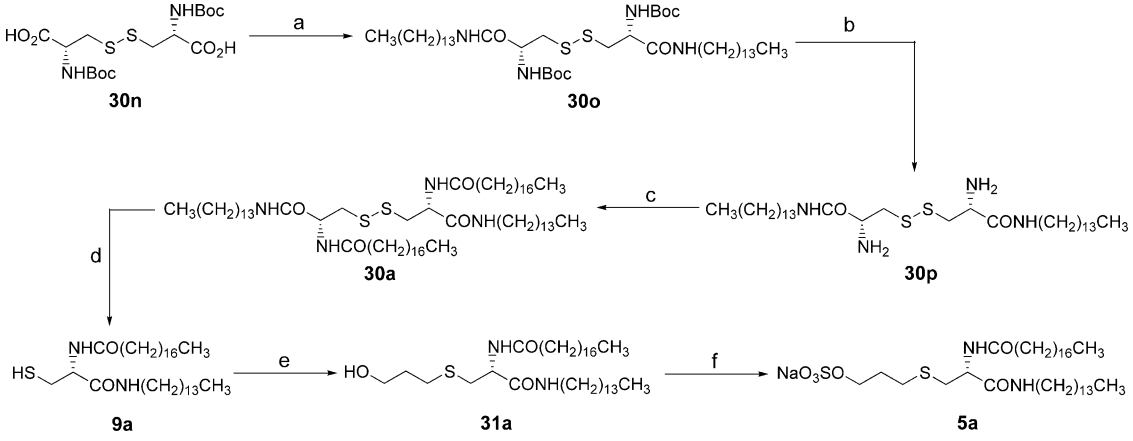
Scheme 2. Reagents: (a) H_2NR^2, EDCI, HOBt; (b) TFA; (c) $ClCOR^1$, iPr_2EtN; (d) Pd/C (10%), H_2; (e) (i) $ClSO_3H$, pyridine; (ii) Na_2CO_3.



Scheme 3. Reagents: (a) $H_2N(CH_2)_{13}CH_3$, EDCI, HOBt; (b) TFA; (c) $ClCO(CH_2)_{16}CH_3$, iPr_2EtN.



Scheme 4. Reagents: (a) Pd/C (10%), H_2; (b) (i) $ClSO_3H$, pyridine; (ii) Na_2CO_3; (c) $NaBH_4$, MeOH; (d) mCPBA.



Scheme 5. Reagents: (a) $H_2N(CH_2)_{13}CH_3$, EDCI, HOBt; (b) TFA; (c) $ClCO(CH_2)_{16}CH_3$, iPr_2EtN; (d) *n*-Bu_3P; (e) K_2CO_3, $BrCH_2CH_2CH_2OH$; (f) (i) $ClSO_3H$, pyridine; (ii) Na_2CO_3.

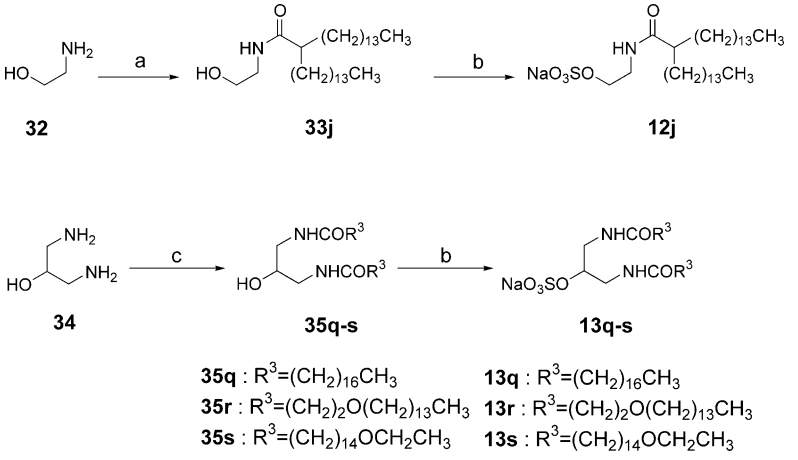
1,3-diamino-2-propanol **34** was acylated, and then followed by sulfation to give the target compounds.

Results and Discussion

For the purpose of monitoring the inhibitory activity of the novel derivatives of sulfatides against acetyl-LDL binding to scavenger receptors, all of them were tested for inhibitory activity against incorporation of 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate-labeled acetyl-LDL (DiI-acetyl-LDL) into macrophages at the concentration of 100 μg/mL or 100 μM at 37 °C. When a tested compound showed more than 40% inhibitory activity, the compound was also tested in lower concentrations. The obtained results are summarized in Tables 1–3.

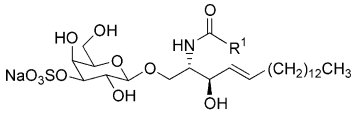
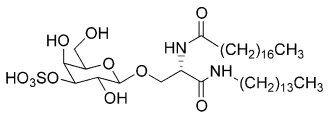
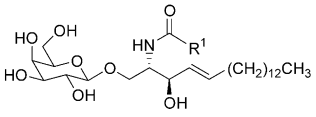
Sulfatides consists of three substructures: sulfate moiety, galactose moiety, and ceramide moiety (Fig. 2). At first, we synthesized a novel derivative in which the ceramide moiety was replaced with a peptide structure bearing two long chains (Table 1, compound **1a**). We chose octadecanoyl group [$CO(CH_2)_{16}CH_3$] and tetradecyl group [$(CH_2)_{13}CH_3$] as two long chains of **1a**. The peptide derivative **1a** was slightly less potent than sulfatides, but it showed a moderate inhibitory activity. We concluded that the ceramide moiety can be replaced with the peptide structure.

Next, compound **2a** which has a phenylene moiety instead of the galactose moiety was prepared (Table 2). It exhibited almost the same potent activity as **1a**, which means that the galactose moiety is not necessary for a potent inhibitory activity, and it can be replaced with another structure like the phenylene moiety. The effect of the 4-ethylphenyl moiety of **2a** on the inhibitory activity was investigated next (Table 2). Ethyl sulfate **3a** and butyl sulfate **4a** showed a potent inhibitory activity comparable to that of **2a**. The potency of 3-ethylthiopropyl sulfate **5a** was weak compared to that of **2a**. These results indicate that the ethylphenyl group of **2a** can be replaced with another group which is not so long as 3-ethylthiopropyl group.



Scheme 6. Reagents: (a) $ClCOCH[(CH_2)_{13}CH_3]_2$, iPr_2EtN; (b) (i) $ClSO_3H$, (ii) Na_2CO_3; (c) $ClCOR^3$, Et_3N.

Table 1. Effects of sulfatides and its related compounds on incorporation of DiI-acetyl-LDL into macrophages

Compound	Structure	Inhibitory activity against incorporation of DiI-acetyl-LDL (%)[a]			
		100 μg/mL	30 μg/mL	10 μg/mL	3 μg/mL
Sulfatides		83.2(3)	77.4(3)	56.2(3)	<20(3)
1a		99.7(3)	63.1(2)	<20(3)	NT[b]
Galactosylceramide		<20(1)	NT	NT	NT

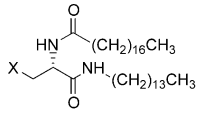
[a]The number on the left side of parentheses represents% inhibition at each concentration. The number inside parentheses represents the number of experiment.
[b]NT, not tested.

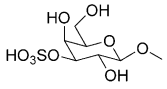
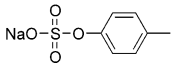
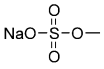
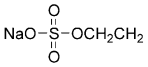
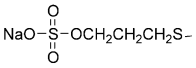
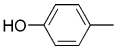
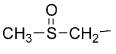
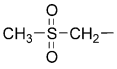
Our next interest was focussed on the effect of the sulfate group of **3a** on the inhibitory activity. Interestingly, the carboxylic acid derivative **6a** did not show a potent inhibitory activity at 100 μM (Table 2), even though maleyl-LDL bearing carboxylic acids as negatively charged functional groups have been known to be a scavenger receptor inhibitor.[17] Galactosylceramide (Table 1), the phenol derivative **7a**, and the hydroxy derivative **8a** (Table 2) exhibited a weak inhibitory activity of less than 30%. Conversion of the sulfate group of **3a** into another sulfur-containing functional group did not lead to the discovery of compounds with more potent inhibitory activity (**9a**, **10a**, and **11a**). We can summarize these results as follows; among the functional groups investigated here, sulfate group was the most preferable for a potent inhibitory activity against incorporation of DiI-acetyl-LDL into macrophages.

Then, in order to elucidate whether the stereochemistry of the asymmetric carbon of **3a** is critical for the inhibitory activity, the enantiomer of **3a** (compound **3b**) was evaluated for the inhibitory activity. As shown in Table 3, **3b** showed almost the same inhibitory activity as that of **3a**, meaning that the stereochemistry of the asymmetric carbon does not affect the inhibitory activity.

Next, we investigated the modification of the two long chains of **3a**. The replacement of the octadecanoyl group with a shorter group, such as acetyl or decanoyl, weakened the potency (**3a** vs **3c**, **3d**). The conversion of the tetradecyl group into propyl group also caused a

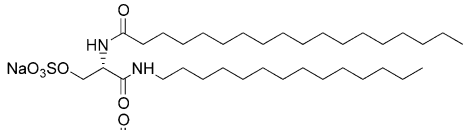
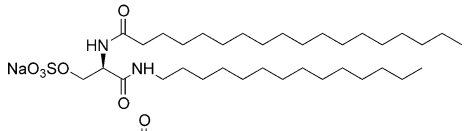
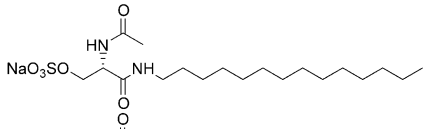
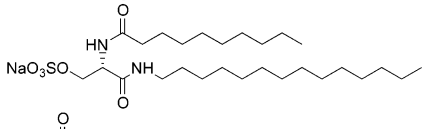
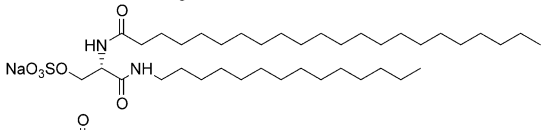
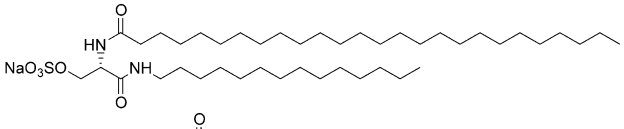
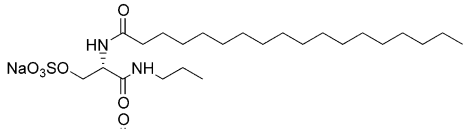
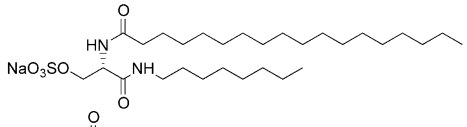
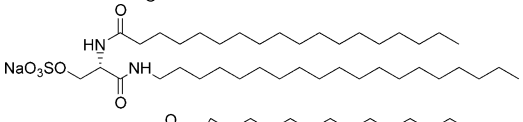
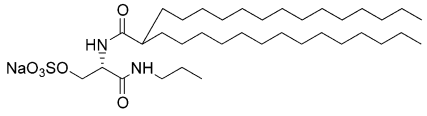
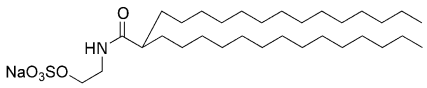
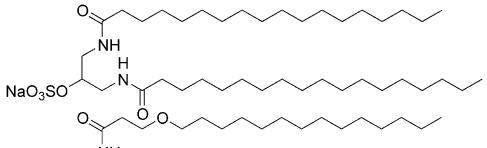
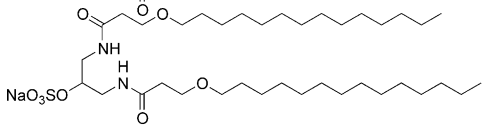
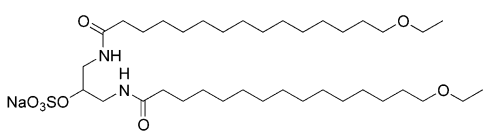
Table 2. Effects of compound **1a** and its related compounds on incorporation of DiI-acetyl-LDL into macrophages



Compd	X	Inhibitory activity against incorporation of DiI-acetyl-LDL(%)[a]		
		100 μM	30 μM	10 μM
1a		99.7(3)[b] (124 μM)	63.1(2)[c] (37 μM)	<20(3)[d] (12.4 μM)
2a		69.5(2)	55.2(2)	<20(1)
3a		61.4(5)	43.8(4)	20.5(2)
4a		62.7(3)	36.3(2)	<20(1)
5a		32.0(1)	NT[e]	NT[e]
6a	HO_2CCH_2—	<20(1)	NT[e]	NT[e]
7a		<20(1)	NT[e]	NT[e]
8a	HO—	27.3(1)	NT[e]	NT[e]
9a	HS—	39.1(1)	NT[e]	NT[e]
10a		20.4(1)	NT[e]	NT[e]
11a		25.2(1)	NT[e]	NT[e]

[a]The number on the left side of parentheses represents % inhibition at each concentration. The number inside parentheses represents the number of experiment.
[b]% inhibition at 124 μM (100 μg/mL).
[c]% inhibition at 37 μM (30 μg/mL).
[d]% inhibition at 12.4 μM (10 μg/mL).
[e]NT, not tested.

Table 3. Effects of compound **3a** and its related compounds on incorporation of DiI-acetyl-LDL into macrophages

Compound	Structure	Inhibitory activity against incorporation of DiI-acetyl-LDL(%)[a]		
		100 μM	3 μM	10 μM
3a		61.4(5)	43.8(4)	20.5(2)
3b		57.1(4)	49.5(2)	28.7(1)
3c		< 20(1)	NT[b]	NT[b]
3d		31.4(2)	NT[b]	NT[b]
3e		65.2(1)	NT[b]	NT[b]
3f		< 20(1)	NT[b]	NT[b]
3g		< 20(1)	NT[b]	NT[b]
3h		45.4(1)	NT[b]	NT[b]
3i		57.4(2)	63.1(2)	23.6(1)
3j		25.5(1)	NT[b]	NT[b]
12j		< 20(1)	NT[b]	NT[b]
13q		60.7(3)	42.8(2)	NT[b]
13r		< 20(1)	NT[b]	NT[b]
13s		< 20(1)	NT[b]	NT[b]

[a]The number on the left side of parentheses represents % inhibition at each concentration. The number inside parentheses represents the number of experiment.
[b]NT, not tested.

reduction in the inhibitory activity (**3a** vs **3g**). The docosanoyl derivative **3e** and the nonadecyl derivative **3i** were as potent as **3a**, while the hexacosanoyl derivative **3f** was less potent than **3a**. These results indicate that the existence of two long chains which consist of **14–22** carbons is necessary for a potent inhibitory activity.

It was noteworthy that both compounds **3j** and **12j** having two long chains (tetradecyl and hexadecanoyl) were weak inhibitors, while compound **13q** with another type of diamide structure was a potent inhibitor. These results means that the existence of two long chains like tetradecyl is necessary, but not enough for a potent inhibitory activity, and another unknown factor is also important. The replacement of methylene group in the two octadecanoyl groups of **13q** with oxygen atom resulted in a reduction in the inhibitory activity (**13r** and **13s**). This result means that the lipophilic property of the two long chains is important for the potent inhibitory activity. The SARs of sulfatides as a scavenger receptor inhibitor are summarized in Figure 3.

Compounds which can inhibit the endocytosis of the formed receptor–ligand complex or inhibit the degradation of acetyl-LDL in the lisosomes are thought to be able to exhibit the inhibitory activity against incorporation of DiI-acetyl-LDL into macrophages. In order to confirm that the inhibitory activity of **3a** against incorporation of DiI-acetyl-LDL into macrophages is based on the inhibition of the binding of DiI-acetyl-LDL to the surface of macrophages, **3a** was examined for the inhibitory activity against the binding of ^{125}I-acetyl-LDL to macrophages at 4 °C (the binding of acetyl-LDLs to the surface of macrophages can occur at 4 °C, but the incorporation of acetyl-LDLs into macrophages can not do at 4 °C). Compound **3a** and sulfatides exhibited the inhibitory activity of 42% at 10 μM (6.7 μg/mL) and 54% at 3 μg/mL, respectively (Table 4). This result means that (1) **3a** has the inhibitory activity against the binding of ^{125}I-acetyl-LDL to macrophage, (2) the inhibitory activity of **3a** against DiI-acetyl-LDL incorporation is based on the inhibition of binding of acetyl-LDL to the surface of macrophages in the same manner as sulfatides. Acetyl-LDL is known to bind to scavenger receptors expressed on the surface of macrophages, so it was thought that **3a** showed its inhibitory activity of incorporation of DiI-acetyl-LDL into macrophages by inhibiting the binding of DiI-acetyl-LDL to scavenger receptors.

Kodama et al. found out that Type I and II class A scavenger receptors are comprised of six domains;[6,7] N-terminal cytoplasmic domain, transmembrane domain, spacer domain, coiled-coil domain, collagen-like domain, and cysteine-rich domain. As a result of stepwise deletion of Type I and II class A scavenger receptors, Doi et al. found that, among the six domains, acetyl-LDL binds to the collagen-like domain.[23] Collagen-like domain in human Type I and II class A scavenger receptors consist of 69 residue sequence bearing glycine (Gly) as every third residue, that is, $(Gly\text{-}X\text{-}Y)_{23}$. Lysine residues which are located in the Y positions of Gly-X-Y triplets near the C-terminus have been reported to be implicated in the ligand binding. We speculated that the sulfate moiety of **3a** or **13q** would interact withε-amino group of one of such lysine residues, and inhibit the binding of acetyl-LDL.

Recently, class A scavenger receptor/apolipoprotein E double-knockout mice were generated by Suzuki et al.,[16] and they reported that, (1) degradation of acetyl-LDL in the macrophages from double-knockout mice was reduced to less than a third compared to that in wild-type mice macrophages, (2) oxidized-LDL degradation activity of the macrophages from double-knockout mice was 50% of that of wild-type mice macrophages, (3) the size of atherosclerotic lesions in double-knockout mice were 60% smaller than that in apolipoprotein E knockout mice.[16] These results are direct evidence that class A scavenger receptors plays an important role in the formation of atherosclerotic lesions. Therefore, **3a** and **13q** with class A scavenger receptor inhibitory activity are expected to prevent the formation of atherosclerosis lesions and become a medicine for the treatment of atherosclerosis.

The reports on the profile of the double-knockout mice by Suzuki et al. shows that class A scavenger receptors are one of promising drug targets for atherosclerosis. However, in spite of the disruption of class A scavenger receptor gene, the atherosclerotic lesions did not disappear completely. This indicates that other scavenger receptors, such as CD36,[9] SR-B1,[11] and LOX-1,[14] may

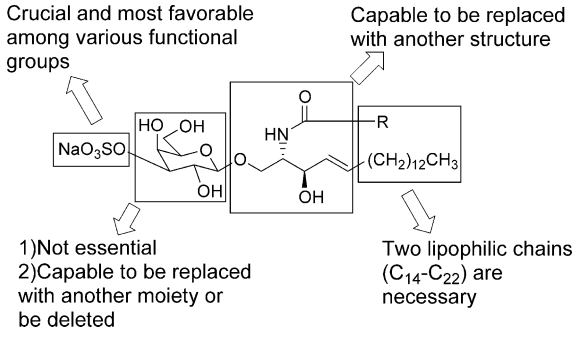


Figure 3. SARs of sulfatides.

Table 4. Effects of compound **3a** and sulfatides on binding of ^{125}I-acetyl-LDL to macrophages

Compound	Concentration	Inhibitory activity against binding of ^{125}I-acetyl-LDL(%)[a]
3a	30 μM (20 μg/mL)	81.1(2)
	10 μM (6.7 μg/mL)	41.8(2)
	3 μM (2.0 μg/mL)	6.6(2)
Sulfatides	10 μg/mL	95.1(3)
	3 μg/mL	54.3(3)
	1 μg/mL	5.9(3)

[a]The number on the left side of parentheses represents % inhibition at each concentration. The number inside parentheses represents the number of experiment.

also participate in the progress of atherosclerosis. The inhibitory activities of **3a** and **13q** against these receptors will be evaluated.

Conclusion

In conclusion, we clarified the structure–activity relationships of sulfatides as a scavenger receptor inhibitor. The obtained information would be very useful for design of effective scavenger receptor inhibitors. In addition, we succeeded in the discovery of novel scavenger receptor inhibitors, such as **3a** and **13q**, which were synthesized easily compared to sulfatides. Further pharmacological evaluation about compounds **3a** and **13q** is in progress, and the results will be reported in due course.

Experimental

Melting points were determined on a capillary melting point apparatus (Yamato MR-21) and are uncorrected. ^{1}H NMR spectra of all the compounds synthesized here were determined with Brucker DPX-250 at 250 MHz (tetramethylsilane as an internal standard). In NMR description, s, d, t, dd, ddd, td, m, br, and br s mean singlet, doublet, triplet, double doublet, double double doublet, triple doublet, multiplet, broad peak, and broad singlet, respectively. Elemental analysis data were obtained by use of Yanagimoto CHNcorder MT-5). Column chromatography was performed using silica gel (YMC-GEL SIL-60A) under medium pressure. No attempt was made to maximize the yields.

Benzyl

(*S*)-2-*tert*-Butoxycarbonylamino-3-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)oxypropionate (17). A mixture of *N*-*tert*-butoxycarbony-L-serine benzyl ester (**16**) (0.72 g, 2.44 mmol), silver carbonate (0.34 g, 1.22 mmol), silver perchlorate (0.26 g, 1.22 mmol) and molecular sieves 4Å (1.00 g), dry dichloromethane (5 mL) was stirred at room temperature for 5 h. A mixture of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**15**) (0.50 g, 1.22 mmol), molecular sieves 4Å (1.00 g), and dry dichloromethane (3 mL), which had been stirred for 5 h, was added to the above mixture, and then the resulting mixture was stirred at room temperature for 18 h. Insoluble matter in the reaction mixture was removed by Celite-filtration, and then the obtained filtrate was concentrated in vacuo. The obtained oil was purified by column chromatography (eluent *n*-hexane/ethyl acetate = 2:1) to give the title compound as a colorless oil (0.16 g, yield = 21%).

^{1}H NMR ($CDCl_3$) δ: 1.42 (s, 9H), 1.95 (s, 3H), 2.00 (s, 3H), 2.05 (s, 3H), 2.11 (s, 3H), 3.75–3.90 (m, 2H), 4.00–4.15 (m, 2H), 4.20–4.35 (m, 1H), 4.35–4.50 (m, 2H), 4.96 (dd, J = 3.4, 10.5 Hz, 1H), 5.05–5.25 (m, 3H), 5.25–5.40 (m, 2H), 7.33 (s, 5H). Anal. calcd for $C_{29}H_{39}NO_{14}$: C, 55.67; H, 6.28; N, 2.24; found: C, 55.87; H, 6.25; N, 2.29.

Benzyl

(*S*)-2-Amino-3-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)oxypropionate (18). A solution of benzyl (*S*)-2-*tert*-butoxycarbonylamino-3-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)oxypropionate (**17**) (2.88 g, 4.60 mmol) in TFA (10 m) was stirred for 3 h under ice-bath cooling. The reaction mixture was poured into a mixture of ethyl acetate (100 mL) and an aqueous solution of sodium hydrogen carbonate (100 mL) under ice-bath cooling. The resulting aqueous layer was extracted with ethyl acetate, and then the organic layers were combined, washed with an aqueous solution of sodium chloride, dried over magnesium sulfate, and concentrated in vacuo to give a colorless oil. Purification of the oil by column chromatography (eluent chloroform/methanol = 50:1) gave the title compound as a colorless oil (1.28 g, yield = 53%).

^{1}H NMR ($CDCl_3$) δ : 1.72 (br s, 2H), 1.99 (s, 3H), 2.04 (s, 3H), 2.07 (s, 3H), 2.15 (s, 3H), 3.66 (t, J = 4.4 Hz, 1H), 3.75–3.95 (m, 2H), 4.10–4.25 (m, 3H), 4.54 (d, J = 7.9 Hz, 1H), 5.00 (dd, J = 3.4, 10.5 Hz, 1H), 5.18 (s, 2H), 5.20 (dd, J = 7.9, 10.5 Hz, 1H), 5.38 (d, J = 3.4 Hz, 1H), 7.38 (s, 5H). Anal. calcd for $C_{24}H_{31}NO_{12}$: C, 54.85; H, 5.95; N, 2.67; found: C, 55.01; H, 5.79; N, 2.77.

Benzyl

(*S*)-2-Hexadecanoylamino-3-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)oxypropionate (19). A solution of octadecanoyl chloride (0.72 g, 2.36 mmol) in *N*,*N*-dimethylformamide (DMF, 10 mL) was added at 0 °C slowly to a solution of benzyl (*S*)-2-amino-3-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)oxypropionate (**18**) (1.18 g, 2.25 mmol) and triethylamine (0.46 g, 4.54 mmol) in DMF (10 mL). The resulting mixture was stirred at 0 °C for 2 h. The reaction mixture was diluted with water (200 mL), and then the whole was extracted with diethyl ether. The organic layer was dried over magnesium sulfate and concentrated in vacuo to give a colorless oil. The oil was purified with column chromatography (eluent first, *n*-hexane/ethyl acetate = 3:1, then 1:1) to give the title compound as a colorless oil (1.52 g, yield = 85%).

^{1}H NMR ($CDCl_3$) δ: 0.88 (t, J = 6.6 Hz, 3H), 1.20–1.40 (m, 28H), 1.55–1.75 (m, 2H), 1.99 (s, 3H), 2.05 (s, 3H), 2.07 (s, 3H), 2.16 (s, 3H), 2.25 (t, J = 7.6 Hz, 2H), 3.82 (t, J = 7.4 Hz, 1H), 3.91 (dd, J = 3.5, 10.5 Hz, 1H), 4.05–4.20 (m, 2H), 4.29 (dd, J = 3.1, 10.5 Hz, 1H), 4.44 (d, J = 7.9 Hz, 1H), 4.65–4.90 (m, 1H), 4.97 (dd, J = 3.4, 10.5 Hz, 1H), 5.16 (dd, J = 7.9, 10.5 Hz, 1H), 5.20 (s, 2H), 5.37 (dd, J = 1.0, 3.4 Hz, 1H), 6.30 (d, J = 7.6 Hz, 1H), 7.25–7.45 (m, 5H). Anal. calcd for $C_{42}H_{65}NO_{13}$: C, 63.70; H, 8.27; N, 1.77; found: C, 63.99; H, 8.35; N, 1.79.

(*S*)-2-Hexadecanoylamino-3-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)oxypropionic acid (20). A mixture of Pd–C (10%, 0.50 g) and dioxane (1 mL) was added to a solution of benzyl (*S*)-2-hexadecanoylamino-3-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)oxypropionate (**19**)

(1.40 g, 1.77 mmol) in tetrahydrofuran (THF, 10 mL), and then the resulting mixture was stirred at room temperature for 2 h under hydrogen atmosphere. Pd/C was removed by filtration, and then the obtained filtrate was concentrated in vacuo to give a brown oil. Purification of the oil by column chromatography (eluent *n*-hexane/ethyl acetate = 1:1) gave the title compound as a colorless solid (1.19 g, yield = 96%).

^{1}H NMR ($CDCl_3$) δ: 0.89 (t, J = 6.6 Hz, 3H), 1.20–1.40 (m, 28H), 1.55–1.75 (m, 2H), 2.00 (s, 3H), 2.07 (s, 3H), 2.09 (s, 3H), 2.17 (s, 3H), 2.28 (t, J = 7.6 Hz, 2H), 3.91 (td, J = 1.0, 6.5 Hz, 1H), 4.00 (dd, J = 4.4, 10.9 Hz, 1H), 4.05–4.30 (m, 3H), 4.53 (d, J = 7.9 Hz, 1H), 4.65–4.75 (m, 1H), 5.01 (dd, J = 3.4, 10.4 Hz, 1H), 5.18 (dd, J = 7.8, 10.4 Hz, 1H), 5.40 (dd, J = 1.0, 3.4 Hz, 1H), 6.43 (d, J = 7.1 Hz, 1H). Anal. calcd for $C_{35}H_{59}NO_{13}$: C, 59.90; H, 8.47; N, 2.00; found: C, 60.06; H, 8.44; N, 2.15.

***N*-[(*S*)-1-Tetradecylcarbamoyl-2-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)oxyethyl]hexadecanamide (21).** EDCI (75 mg, 0.39 mmol) and tetradecylamine (77 mg, 0.36 mmol) were added at 0 °C to a solution of (*S*)-2-hexadecanoylamino-3-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)oxypropionic acid (**20**) (0.25 g, 0.36 mmol) and HOBt (53 mg, 0.39 mmol) in DMF (5 mL), and then the resulting mixture was stirred at room temperature for 19 h. The reaction mixture was diluted with water (30 mL), and then the whole was extracted with ethyl acetate. Combined organic layer was washed with water and brine, and dried over magnesium sulfate. Concentration of the solution under reduced pressure gave a colorless oil. Purification of the oil by column chromatography (eluent *n*-hexane/ethyl acetate = 1:1) gave the title compound as a colorless powder (53 mg, yield = 16%).

^{1}H NMR ($CDCl_3$) δ: 0.89 (t, J = 6.6 Hz, 6H), 1.20–1.40 (m, 50H), 1.40–1.80 (m, 4H), 2.00 (s, 3H), 2.06 (s, 3H), 2.09 (s, 3H), 2.16 (s, 3H), 2.25 (t, J = 7.5 Hz, 2H), 3.15–3.40 (m, 2H), 3.81 (dd, J = 7.9, 9.5 Hz, 1H), 3.92 (td, J = 1.0, 6.7 Hz, 1H), 3.99 (dd, J = 3.6, 9.5 Hz, 1H), 4.05–4.20 (m, 2H), 4.30–4.45 (m, 1H), 4.59 (d, J = 7.5 Hz, 1H), 5.05 (dd, J = 3.3, 10.6 Hz, 1H), 5.15 (dd, J = 7.5, 10.6 Hz, 2H), 5.42 (dd, J = 1.0, 3.3 Hz, 1H), 6.43 (t, J = 5.5 Hz, 1H), 6.51 (d, J = 6.6 Hz, 1H). Anal. calcd for $C_{49}H_{88}N_2O_{12}$: C, 65.59; H, 9.89; N, 3.12; found: C, 65.77; H, 10.01; N, 3.11.

***N*-[(*S*)-1-Tetradecylcarbamoyl-2-(β-D-galactopyranosyl)-oxyethyl]hexadecanamide (22).** A solution of sodium methoxide in methanol (28%, 165 mg, 0.854 mmol) was added at 0 °C to a solution of *N*-[1(*S*)-tetradecylcarbamoyl-2-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)oxyethyl] hexadecanamide (**21**) (174 mg, 0.194 mmol) in THF (10 mL) and methanol (1 mL), and then the resulting mixture was stirred at room temperature for 3 h. Diluted hydrochloric acid was added to the reaction mixture until the pH of the mixture showed 7. The resulting mixture was diluted with water (30 mL), and the precipitated solid was collected by filtration to give the title compound as a colorless powder (90 mg, yield = 64%).

^{1}H NMR (CD_3OD) δ: 0.90 (t, J = 6.6 Hz, 6H), 1.20–1.40 (m, 50H), 1.40–1.75 (m, 4H), 2.23 (t, J = 7.3 Hz, 2H), 3.15–3.30 (m, 2H), 3.40–4.60 (m, 10H), 4.57 (br s, 6H). Anal. calcd for $C_{41}H_{80}N_2O_8$: C, 67.54; H, 11.06; N, 3.84; found: C, 67.55; H, 10.94; N, 3.55.

***N*-[(*S*)-1-Tetradecylcarbamoyl-2-(3-sulfo-β-D-galactopyranosyl)oxyethyl]hexadecanamide (1a).** A mixture of *N*-[(*S*)-1-tetradecylcarbamoyl-2-(β-D-galactopyranosyl)oxyethyl]hexadecanamide (**22**) (110 mg, 0.151 mmol), di-*n*-butyltin oxide 38 mg (38 mg, 0.151 mmol), methanol (20 mL) was refluxed for 3 h. The solvent was removed, and then a mixture of sulfur trioxide–trimethylamine complex (42 mg, 0.302 mmol) and dioxane (20 mL) was added to the obtained residue. The resulting mixture was stirred at room temperature for 65 h. Methanol (20 mL) was added to the reaction mixture, and then insoluble matter was collected by filtration to give a colorless powder. Purification of this crude product of the title compound by recrystallization from acetonitrile and the subsequent preparative TLC (developing solvent/chloroform:methanol = 4:1) gave the title compound as a colorless solid (7 mg, yield = 5.7%).

^{1}H NMR (CD_3OD) δ: 0.91 (t, J = 6.6 Hz, 6H), 1.20–1.45 (m, 50H), 1.45–1.80 (m, 4H), 2.20–2.40 (m, 2H), 3.10–3.30 (m, 2H), 3.55–4.65 (m, 10H), 4.84 (br s, 6H). Anal. calcd for $C_{41}H_{80}N_2O_{11}S{\cdot}1.0H_2O$: C, 59.53; H, 9.99; N, 3.39; found: C, 59.31; H, 10.20; N, 3.59.

***tert*-Butyl *N*-[(*S*)-2-benzyloxy-1-(tetradecylcarbamoyl)-ethyl]carbamate (24a).** EDCI (9.34 g, 48.7 mmol) and HOBt (6.58 g, 48.7 mmol) were added to a mixture of *N*-Boc-*O*-benzyl-L-serine (12.0 g, 40.6 mmol), tetradecylamine (10.4 g, 48.7 mmol), and DMF under ice-cooling, and the resulting mixture was stirred at room temperature for 20 h. Chloroform (400 mL) was added to the reaction mixture, and then the resulting mixture was washed with water, a saturated aqueous solution of sodium hydrogen carbonate, and a saturated aqueous solution of sodium chloride, dried over magnesium sulfate, and concentrated in vacuo. The obtained residue was purified with column chromatography (eluent; chloroform) to give the title compound as a colorless powder (19.3 g, yield = 97%). Recrystallization from a mixture of methanol and water gave an analytical sample having the following physical chemical properties.

Mp 57.0–57.5 °C. ^{1}H NMR ($CDCl_3$) δ: 0.88 (t, J = 6.6 Hz, 3H), 1.10–1.35 (m, 22H), 1.45 (s, 9H), 1.35–1.65 (m, 2H), 3.20–3.35 (m, 2H), 3.56 (dd, J = 6.7, 9.2 Hz, 1H), 3.91 (dd, J = 4.0, 9.2 Hz, 1H), 4.10–4.30 (m, 1H), 4.50 (d, J = 11.8 Hz, 1H), 4.59 (d, J = 11.8 Hz, 1H), 5.25–5.50 (br, 1H), 6.30–6.50 (br, 1H), 7.20–7.40 (m, 5H). Anal. calcd for $C_{29}H_{50}N_2O_4$: C, 70.98; H, 10.27; N, 5.71; found: C, 71.00; H, 10.31; N, 5.67.

(*S*)-2-Amino-3-benzyloxy-*N*-tetradecylpropionamide (25a). A mixture of *tert*-butyl *N*-[(*S*)-2-benzyloxy-1-(tetradecylcarbamoyl)ethyl]carbamate (**24**) (19.0 g, 38.7 mmol) and TFA (70 mL) was stirred 1 h for under ice-cooling. The reaction mixture was poured into a mixture of a

saturated aqueous solution of sodium hydrogen carbonate and ethyl acetate. The organic layer was washed with a saturated aqueous solution of sodium hydrogen carbonate and a saturated aqueous solution of sodium chloride, dried over magnesium sulfate, and concentrated in vacuo. The obtained residue was purified with column chromatography (eluent *n*-hexane/ethyl acetate = 1:1) to give the title compound as a colorless powder (11.3 g, yield = 75%). Recrystallization from ethyl acetate gave an analytical sample having the following physical chemical properties.

Mp 60.5–62.5 °C. ^{1}H NMR ($CDCl_3$) δ: 0.88 (t, J = 6.6 Hz, 3H), 1.15–1.40 (m, 22H), 1.40–1.60 (m, 2H), 1.64 (br s, 2H), 3.15–3.30 (m, 2H), 3.58 (dd, J = 3.8, 6.6 Hz, 1H), 3.64 (dd, J = 6.6, 8.8 Hz, 1H), 3.75 (dd, J = 3.8, 8.8 Hz, 1H), 4.54 (s, 2H), 7.20–7.45 (m, 6H). Anal. calcd for $C_{24}H_{42}N_2O_2$: C, 73.80; H, 10.84; N, 7.17; found: C, 73.86; H, 10.83; N, 7.25.

***N*-[(*S*)-2-Benzyloxy-1-(tetradecylcarbamoyl)ethyl]octadecanamide (26a).** A solution of octadecanoyl chloride (1.71 g, 5.64 mmol) in THF (5 mL) was added dropwise to a mixture of (*S*)-2-amino-3-benzyloxy-*N*-tetradecylpropionamide (**25**) (2.00 g, 5.12 mmol), diisopropylethylamine (660 mg, 5.11 mmol), and THF (15 mL) under ice-cooling, and then the resulting mixture was stirred at room temperature for 18 h. After the reaction mixture had been concentrated in vacuo, chloroform (50 mL) was added to the obtained residue. The resulting mixture was washed with a saturated aqueous solution of sodium chloride, dried over magnesium sulfate and concentrated in vacuo. The obtained residue was purified with column chromatography (eluent *n*-hexane/ethyl acetate = 2:1) to give the title compound as a brown powder (2.38 g, yield = 71%). Recrystallization from ethyl acetate gave an analytical sample having the following physical chemical properties.

Mp 87.0–89.0 °C. ^{1}H NMR ($CDCl_3$) δ: 0.88 (t, J = 6.6 Hz, 6H), 1.20–1.40 (m, 50H), 1.40–1.70 (m, 4H), 2.21 (t, J = 7.6 Hz, 2H), 3.15–3.30 (m, 2H), 3.48 (dd, J = 8.0, 9.2 Hz, 1H), 3.89 (dd, J = 4.2, 9.2 Hz, 1H), 4.45–4.60 (m, 1H), 4.53 (d, J = 11.7 Hz, 1H), 4.62 (d, J = 11.7 Hz, 1H), 6.30–6.45 (br, 2H), 7.20–7.45 (m, 5H). Anal. calcd for $C_{42}H_{76}N_2O_3$: C, 76.77; H, 11.66; N, 4.26; found: C, 76.78; H, 11.63; N, 4.30.

The following compounds **10a**, **26b**, **27a**, **28a**, and **30a** were synthesized from *N*-Boc-*O*-benzyl-D-serine, *N*-Boc-L-methionine sulfoxide (**10n**), *N*-Boc-L-glutamic acid 5-benzyl ester (**27n**), *N*-Boc-*O*-benzyl-L-tyrosine (**28n**), and *N*-Boc-L-cystine (**30n**), respectively, in the same manner as the synthesis of **26a**.

***N*-[(*S*)-3-Methanesulfinyl-1-(tetradecylcarbamoyl)propyl]-octadecanamide (10a).** Mp 106.5–108.5 °C. ^{1}H NMR ($CDCl_3$) δ: 0.88 (t, J = 6.6 Hz, 6H), 1.10–1.40 (m, 50H), 1.40–1.70 (m, 4H), 2.10–2.55 (m, 4H), 2.58 (s), 2.67 (s), 2.70–3.00 (m, 2H), 3.15–3.30 (m, 2H), 4.50–4.70 (m, 1H), 6.75–7.15 (br, 2H). Anal. calcd for $C_{37}H_{74}N_2O_3S$: C, 70.87; H, 11.89; N, 4.47; found: C, 70.88; H, 11.93; N, 4.23.

***N*-[(*R*)-2-Benzyloxy-1-(tetradecylcarbamoyl)ethyl]octadecanamide (26b).** Mp 87.0–89.0 °C. ^{1}H NMR ($CDCl_3$) δ: 0.88 (t, J = 6.6 Hz, 6H), 1.20–1.40 (m, 50H), 1.40–1.70 (m, 4H), 2.21 (t, J = 7.6 Hz, 2H), 3.15–3.30 (m, 2H), 3.48 (dd, J = 8.0, 9.2 Hz, 1H), 3.89 (dd, J = 4.2, 9.2 Hz, 1H), 4.45–4.60 (m, 1H), 4.53 (d, J = 11.7 Hz, 1H), 4.62 (d, J = 11.7 Hz, 1H), 6.30–6.45 (br, 2H), 7.20–7.45 (m, 5H). Anal. calcd for $C_{42}H_{76}N_2O_3$: C, 76.77; H, 11.66; N, 4.26; found: C, 76.99; H, 11.65; N, 4.26.

Benzyl (*S*)-4-tetradecylcarbamoyl-4-octadecanoylaminobutanoate (27a). Mp 93.5–94.5 °C. ^{1}H NMR ($CDCl_3$) δ: 0.88 (t, J = 6.5 Hz, 6H), 1.15–1.40 (m, 50H), 1.40–1.75 (m, 4H), 1.85–2.20 (m, 4H), 2.30–2.70 (m, 2H), 3.10–3.30 (m, 2H), 4.35–4.50 (m, 1H), 5.10 (d, J = 12.3 Hz, 1H), 5.16 (d, J = 12.3 Hz, 1H), 6.25–6.45 (br, 2H), 7.25–7.45 (m, 5H). Anal. calcd for $C_{44}H_{78}N_2O_4$: C, 75.59; H, 11.25; N, 4.01; found: C, 75.57; H, 11.,25; N, 3.78.

***N*-[(*S*)-2-(4-Benzyloxy)phenyl-1-(tetradecylcarbamoyl)-ethyl]octadecanamide (28a).** Mp 123.5–125.5 °C. ^{1}H NMR ($CDCl_3$) δ: 0.88 (t, J = 6.6 Hz, 6H), 1.10–1.40 (m, 50H), 1.40–1.80 (m, 4H), 2.16 (t, J = 7.6 Hz, 2H), 2.90 (dd, J = 8.6, 13.7 Hz, 1H), 2.95–3.20 (m, 3H), 4.40–4.60 (m, 1H), 5.03 (s, 2H), 5.55 (br, 1H), 6.14 (d, J = 7.8 Hz, 1H), 6.90 (d, J = 8.7 Hz, 2H), 7.13 (d, J = 8.7 Hz, 2H), 7.30–7.45 (m, 5H). Anal. calcd for $C_{48}H_{80}N_2O_3$: C, 78.63; H, 11.00; N, 3.82; found: C, 78.60; H, 10.98; N, 3.85.

***N*-[(*R*)-2-[(*R*)-2-Tetradecylcarbamoyl-2-octadecanoylaminoethyl]dithio-1-(tetradecylcarbamoyl)ethyl]octadecanamide (30a).** ^{1}H NMR ($CDCl_3$) δ: 0.88 (t, J = 6.6 Hz, 12H), 1.20–1.40 (m, 100H), 1.40–1.70 (m, 8H), 2.25 (t, J = 7.5 Hz, 4H), 2.86 (dd, J = 10.0, 14.7 Hz, 2H), 2.97 (dd, J = 4.5, 14.7 Hz, 2H), 3.15–3.40 (m, 4H), 5.33 (ddd, J = 4.5, 9.2, 10.0 Hz, 2H), 6.44 (d, J = 9.2 Hz, 2H), 8.17 (t, J = 4.8 Hz, 2H). Anal. calcd for $C_{70}H_{138}N_4O_4S_2$: C, 72.23; H, 11.95; N, 4.81; found: C, 72.21; H, 12.02; N, 4.79.

***N*-[(*S*)-2-Hydroxy-1-(tetradecylcarbamoyl)ethyl]octadecanamide (8a).** A mixture of *N*-[(*S*)-2-benzyloxy-1-(tetradecylcarbamoyl)ethyl]octadecanamide (**26**) (1.40 g, 2.13 mmol), Pd/C (10%, 1.13 g), dioxane (120 mL), and methanol (200 mL) was stirred at room temperature under hydrogen atmosphere for 46 h. After addition of chloroform (100 mL) to the reaction mixture, Pd/C was removed by filtration. The obtained filtrate was concentrated in vacuo to dryness. Ethyl acetate (20 mL) was added to the obtained reside, and then the resulting mixture was stirred. Finally, the insoluble crystalline solid was collected by filtration to give the title compound as a colorless crystalline powder (1.05 g, yield = 87%). Recrystallization from chloroform gave an analytical sample having the following physical chemical properties.

Mp 119.0–121.0 °C. ^{1}H NMR ($CDCl_3$) δ: 0.88 (t, J = 6.6 Hz, 6H), 1.15–1.40 (m, 50H), 1.40–1.75 (m, 4H), 1.90 (br s, 1H), 2.20–2.35 (m, 2H), 3.10–3.35 (m, 2H), 3.60 (dd, J = 4.1, 11.6 Hz, 1H), 4.18 (dd, J = 2.5, 11.6 Hz, 1H), 4.30–4.45 (m, 1H), 6.55–6.75 (br, 1H), 6.75–6.95 (br, 1H). Anal. calcd for $C_{35}H_{70}N_2O_3$: C, 74.15; H, 12.44; N, 4.94; found: C, 74.08; H, 12.48; N, 4.89.

The following compounds **8c–j** were synthesized from *N*-Boc-*O*-benzyl-L-serine and the corresponding alkylamine and acyl chloride via the corresponding *N*-[(*S*)-2-benzyloxy-1-(alkylcarbamoyl)ethyl]alkanamide in the same manner as **8a**.

***N*-[(*S*)-2-Hydroxy-1-(tetradecylcarbamoyl)ethyl]acetamide (8c).** ^{1}H NMR ($CDCl_3$) δ: 0.88 (t, J = 6.6 Hz, 3H), 1.20–1.40 (m, 22H), 1.40–1.60 (m, 2H), 2.05 (s, 3H), 3.23 (td, J = 6.7, 6.7 Hz, 2H), 3.40–3.70 (m, 2H), 4.12 (dd, J = 2.9, 10.9 Hz, 2H), 4.30–4.45 (m, 1H), 6.67 (d, J = 6.9 Hz, 1H), 6.79 (br, 1H). Anal. calcd for $C_{19}H_{38}N_2O_3$: C, 66.63; H, 11.18; N, 8.18; found: C, 66.35; H, 11.18; N, 8.47.

***N*-[(*S*)-2-Hydroxy-1-(tetradecylcarbamoyl)ethyl]decanamide (8d).** Mp 127.0–128.0 °C. ^{1}H NMR ($CDCl_3$) δ: 0.88 (t, J = 6.6 Hz, 6H), 1.15–1.40 (m, 34H), 1.40–1.75 (m, 4H), 2.15–2.30 (m, 3H), 3.15–3.30 (m, 2H), 3.50–3.70 (m, 2H), 4.10–4.20 (m, 1H), 4.30–4.45 (m, 1H), 6.64 (d, J = 7.0 Hz, 1H), 6.83 (br, 1H). Anal. calcd for $C_{27}H_{54}N_2O_3$: C, 71.31; H, 11.97; N, 6.16; found: C, 71.48; H, 11.89; N, 6.11.

***N*-[(*S*)-2-Hydroxy-1-(tetradecylcarbamoyl)ethyl]docosanamide (8e).** Mp 115.0–117.0 °C. ^{1}H NMR ($CDCl_3$) δ: 0.88 (t, J = 6.6 Hz, 6H), 1.15–1.40 (m, 58H), 1.40–1.75 (m, 4H), 2.10 (br s, 1H), 2.15–2.30 (m, 2H), 3.15–3.30 (m, 2H), 3.50–3.70 (m, 1H), 4.10–4.25 (m, 1H), 4.30–4.40 (m, 1H), 6.62 (d, J = 7.3 Hz, 1H), 6.75–6.90 (m, 1H). Anal. calcd for $C_{39}H_{78}N_2O_3$: C, 75.18; H, 12.62; N, 4.50; found: C, 75.21; H, 12.63; N, 4.49.

***N*-[(*S*)-2-Hydroxy-1-(tetradecylcarbamoyl)ethyl]hexacosanamide (8f).** ^{1}H NMR ($CDCl_3$) δ: 0.88 (t, J = 6.6 Hz, 6H), 1.15–1.40 (m, 66H), 1.40–1.75 (m, 4H), 2.20–2.30 (m, 2H), 3.15–3.30 (m, 2H), 3.30–3.45 (m, 1H), 3.50–3.65 (m, 1H), 4.10–4.20 (m, 1H), 4.30–4.40 (m, 1H), 6.57 (d, J = 6.5 Hz, 1H), 6.65–6.85 (m, 1H). Anal. calcd for $C_{43}H_{86}N_2O_3$: C, 76.04; H, 12.76; N, 4.12; found: C, 76.06; H, 12.91; N, 3.98.

***N*-[(*S*)-2-Hydroxy-1-(propylcarbamoyl)ethyl]octadecanamide (8g).** ^{1}H NMR ($CDCl_3$) δ: 0.80–1.00 (m, 6H), 1.10–1.40 (m, 26H), 1.40–1.75 (m, 4H), 2.24 (t, J = 7.5 Hz, 2H), 3.21 (td, J = 6.6, 6.6 Hz, 2H), 3.50–3.70 (m, 1H), 3.81 (br s, 1H), 4.07 (dd, J = 3.0, 11.0 Hz, 1H), 4.35–4.50 (m, 1H), 6.73 (d, J = 6.9 Hz, 1H), 6.99 (br, 1H). Anal. calcd for $C_{24}H_{48}N_2O_3$: C, 69.86; H, 11.72; N, 6.79; found: C, 70.05; H, 11.99; N, 6.78.

***N*-[(*S*)-2-Hydroxy-1-(octylcarbamoyl)ethyl]octadecanamide (8h).** Mp 114.0–115.0 °C. ^{1}H NMR (DMSO-d_6) δ: 0.85 (t, J = 6.6 Hz, 6H), 1.10–1.60 (m, 42H), 2.05–2.20 (m, 2H), 2.90–3.10 (m, 2H), 3.40–3.60 (m, 2H), 4.15–4.30 (m, 1H), 4.77 (t, J = 5.6 Hz, 1H), 7.60–7.80 (m, 2H). Anal. calcd for $C_{29}H_{58}N_2O_3$: C, 72.15; H, 12.11; N, 5.80; found: C, 72.21; H, 11.98; N, 5.80.

***N*-[(*S*)-2-Hydroxy-1-(nonadecylcarbamoyl)ethyl]octadecanamide (8i).** Mp 122.0–124.5 °C. ^{1}H NMR ($CDCl_3$) δ: 0.88 (t, J = 6.6 Hz, 6H), 1.15–1.40 (m, 60H), 1.40–1.80 (m, 5H), 2.20–2.35 (m, 2H), 3.15–3.30 (m, 2H), 3.58 (dd, J = 4.1, 11.5 Hz, 1H), 4.17 (dd, J = 2.3, 11.5 Hz, 1H), 4.30–4.45 (m, 1H), 6.55–6.70 (br, 1H), 6.75–6.90 (br, 1H). Anal. calcd for $C_{40}H_{80}N_2O_3$: C, 75.41; H, 12.66; N, 4.40; found: C, 75.31; H, 12.62; N, 4.27.

***N*-[(*S*)-2-Hydroxy-1-(propylcarbamoyl)ethyl]-2-tetradecylhexadecanamide (8j).** ^{1}H NMR ($CDCl_3$) δ: 0.88 (t, J = 6.6 Hz, 6H), 0.91 (t, J = 7.4 Hz, 3H), 1.10–1.40 (m, 48H), 1.40–1.70 (m, 6H), 2.00–2.20 (m, 1H), 3.10–3.30 (m, 2H), 3.35–3.65 (m, 2H), 4.18 (d, J = 2.6, 11.9 Hz, 1H), 4.30–4.45 (m, 1H), 6.57 (d, J = 7.1 Hz, 1H), 6.80–7.00 (m, 1H). Anal. calcd for $C_{36}H_{72}N_2O_3$: C, 74.42; H, 12.49; N, 4.82; found: C, 74.70; H, 12.55; N, 4.93.

The following compounds **8b** and **7a** were synthesized from the corresponding benzyloxy derivatives **26b** and **28a**, respectively, in the same manner as **8a**.

***N*-[(*R*)-2-Hydroxy-1-(tetradecylcarbamoyl)ethyl]octadecanamide (8b).** Mp 119.5–120.5 °C. ^{1}H NMR ($CDCl_3$) δ: 0.88 (t, J = 6.6 Hz, 6H), 1.15–1.40 (m, 50H), 1.40–1.70 (m, 5H), 2.20–2.30 (m, 2H), 3.15–3.30 (m, 2H), 3.50–3.65 (m, 1H), 4.10–4.25 (m, 1H), 4.30–4.40 (m, 1H), 6.50–6.65 (m, 1H), 6.70–6.85 (br, 1H). Anal. calcd for $C_{35}H_{70}N_2O_3 \cdot 0.25H_2O$: C, 73.56; H, 12.43; N, 4.90; found: C, 73.57; H, 12.39; N, 4.77.

***N*-[(*R*)-2-(4-Hydroxyphenyl)-1-(tetradecylcarbamoyl)-ethyl]octadecanamide (7a).** Mp 134.5–136.5 °C. ^{1}H NMR (DMSO-d_6) δ: 0.85 (t, J = 6.6 Hz, 6H), 1.10–1.60 (m, 54H), 2.02 (t, J = 7.3 Hz, 2H), 2.61 (dd, J = 8.9, 13.7 Hz, 1H), 2.80 (dd, J = 5.7, 13.7 Hz, 1H), 2.90–3.10 (m, 2H), 4.25–4.45 (m, 1H), 6.61 (d, J = 8.5 Hz, 2H), 6.98 (d, J = 8.5 Hz, 2H), 7.70–7.80 (m, 1H), 7.86 (d, J = 8.3 Hz, 1H), 9.08 (s, 1H). Anal. calcd for $C_{41}H_{74}N_2O_3$: C, 76.58; H, 11.60; N, 4.36; found: C, 76.41; H, 11.58; N, 4.50.

(*S*)-2-Octadecanoylamino-2-(tetradecylcarbamoyl)ethyl sulfate sodium salt (3a). Chlorosulfonic acid (70 μL, 1.06 mmol) was added dropwise to pyridine (2 mL) at room temperature with attention, and then the resulting mixture was stirred at 70–75 °C for 30 min. After the reaction mixture had been cooled to room temperature, a solution of *N*-[(*S*)-2-hydroxy-1-(tetradecylcarbamoyl)ethyl]octadecanamide (0.20 g, 0.353 mmol) in pyridine (5 mL) was added to the reaction mixture, and then the resulting mixture was stirred at 70–75 °C for 1 h. After evaporation of pyridine from the reaction mixture in vacuo, methanol (10 mL) was added to the obtained residue. A saturated aqueous solution of sodium carbonate was added to the resulting mixture till pH of the mixture showed 9. Water (10 mL) was added to the alkaline mixture, and then the precipitating solid was collected by filtration, and dried in vacuo. After the obtained solid had been stirred with hot chloroform (40 mL), insoluble matter was removed by filtration. The obtained filtrate was concentrated in vacuo. Finally, the residue was stirred with ethyl acetate (10 mL), and then the precipitating crystals were collected by filtration to give the title compound as a colorless powder (150 mg, Yield = 64%).

Mp 139.0–144.0 °C (dec). ^{1}H NMR ($CDCl_3$) δ: 0.88 (t, J = 6.5 Hz, 6H), 1.10–1.40 (m, 50H), 1.40–1.80 (m, 4H), 2.15–2.30 (m, 2H), 3.00–3.30 (m, 2H), 4.10–4.30 (m, 2H), 4.65–4.80 (m, 1H), 7.20–7.60 (br, 2H). Anal. calcd for $C_{35}H_{69}N_2NaO_6S{\cdot}1.5H_2O$: C, 60.40; H, 10.43; N, 4.02; found: C, 60.57; H, 10.51; N, 4.09.

The following compounds **3b–j** were synthesized from the corresponding hydroxyl derivatives **8b–j** in the same manner as **3a**.

(*R*)-2-Octadecanoylamino-2-(tetradecylcarbamoyl)ethyl sulfate sodium salt (3b). Mp 139.0–144.0 °C (dec). ^{1}H NMR ($CDCl_3$) δ: 0.88 (t, J = 6.5 Hz, 6H), 1.10–1.40 (m, 50H), 1.40–1.80 (m, 4H), 2.15–2.30 (m, 2H), 3.00–3.30 (m, 2H), 4.10–4.30 (m, 2H), 4.65–4.80 (m, 1H), 7.20–7.60 (br, 2H). Anal. calcd for $C_{35}H_{69}N_2NaO_6S{\cdot}1.5H_2O$: C, 60.40; H, 10.43; N, 4.02; found: C, 60.11; H, 10.14; N, 3.70.

(*S*)-2-Acetyamino-2-(tetradecylcarbamoyl)ethyl sulfate sodium salt (3c). Mp 140.0–143.0 °C (dec). ^{1}H NMR ($CDCl_3$) δ: 0.88 (t, J = 6.6 Hz, 3H), 1.15–1.40 (m, 22H), 1.40–1.60 (m, 2H), 2.02 (s, 3H), 3.00–3.35 (m, 2H), 4.10–4.40 (m, 2H), 4.70–4.90 (m, 1H), 7.30–7.90 (br, 2H). Anal. calcd for $C_{19}H_{37}N_2NaO_6S{\cdot}1.5H_2O$: C, 48.39; H, 8.55; N, 5.94; found: C, 48.35; H, 8.35; N, 5.71.

(*S*)-2-Decanoylamino-2-(tetradecylcarbamoyl)ethyl sulfate sodium salt (3d). Mp 140.0–145.0 °C (dec). ^{1}H NMR ($CDCl_3$) δ: 0.88 (t, J = 6.6 Hz, 6H), 1.15–1.40 (m, 34H), 1.40–1.65 (m, 4H), 2.25 (t, J = 7.5 Hz, 2H), 3.00–3.30 (m, 2H), 4.10–4.40 (m, 2H), 4.65–4.85 (m, 1H), 7.20–7.70 (br, 2H). Anal. calcd for $C_{27}H_{53}N_2NaO_6S{\cdot}0.75H_2O$: C, 56.87; H, 9.63; N, 4.91; found: C, 56.92; H, 9.42; N, 4.51.

(*S*)-2-Docosanoylamino-2-(tetradecylcarbamoyl)ethyl sulfate sodium salt (3e). Mp 137.0–140.0 °C (dec). ^{1}H NMR ($CDCl_3$) δ: 0.88 (t, J = 6.6 Hz, 6H), 1.10–1.40 (m, 58H), 1.40–1.70 (m, 4H), 2.10–2.35 (m, 2H), 3.00–3.30 (m, 2H), 4.10–4.40 (m, 2H), 4.65–4.85 (m, 1H), 7.20–7.70 (br, 2H). Anal. calcd for $C_{39}H_{77}N_2NaO_6S{\cdot}1.5H_2O$: C, 62.28; H, 10.72; N, 3.72; found: C, 62.33; H, 10.75; N, 3.68.

(*S*)-2-Hexacosanoylamino-2-(tetradecylcarbamoyl)ethyl sulfate sodium salt (3f). Mp 133.0–134.0 °C (dec). ^{1}H NMR ($CDCl_3$) δ: 0.88 (t, J = 6.6 Hz, 6H), 1.10–1.40 (m, 66H), 1.40–1.70 (m, 4H), 2.15–2.35 (m, 2H), 3.00–3.30 (m, 2H), 4.10–4.40 (m, 2H), 4.60–4.85 (m, 1H), 7.20–7.70 (br, 2H). Anal. calcd for $C_{43}H_{85}N_2NaO_6S{\cdot}2.0H_2O$: C, 63.20; H, 10.98; N, 3.43; found: C, 63.21; H, 10.90; N, 3.14.

(*S*)-2-Octadecanoylamino-2-(propylcarbamoyl)ethyl sulfate sodium salt (3g). Mp 91.5–92.5 °C (dec). ^{1}H NMR (DMSO-d_6) δ: 0.81 (t, J = 7.4 Hz, 3H), 0.85 (t, J = 6.7 Hz, 3H), 1.10–1.60 (m, 32H), 2.11 (t, J = 7.3 Hz, 2H), 2.90–3.05 (m, 2H), 3.84 (d, J = 5.8 Hz, 2H), 4.25–4.40 (m, 1H), 7.74 (t, J = 5.7 Hz, 1H), 7.87 (d, J = 7.9 Hz, 1H). Anal. calcd for $C_{24}H_{47}N_2NaO_6S{\cdot}2.0H_2O$: C, 52.35; H, 9.33; N, 5.08; found: C, 52.64; H, 8.98; N, 5.22.

(*S*)-2-Octadecanoylamino-2-(octylcarbamoyl)ethyl sulfate sodium salt (3h). Mp 120.0–122.0 °C (dec). ^{1}H NMR (DMSO-d_6) δ: 0.85 (t, J = 6.6 Hz, 6H), 1.10–1.60 (m, 42H), 2.05–2.15 (m, 2H), 2.95–3.10 (m, 2H), 3.84 (d, J = 5.9 Hz, 2H), 4.25–4.40 (m, 1H), 7.71 (t, J = 5.4 Hz, 1H), 7.88 (d, J = 8.0 Hz, 1H). Anal. calcd for $C_{29}H_{57}N_2NaO_6S{\cdot}1.5H_2O$: C, 56.93; H, 9.88; N, 4.58; found: C, 56.90; H, 9.65; N, 4.50.

(*S*)-2-Nonadecylcarbamoyl-2-(octadecanoylamino)ethyl sulfate sodium salt (3i). Mp 137.0–139.0 °C (dec). ^{1}H NMR (DMSO-d_6) δ: 0.85 (t, J = 6.6 Hz, 6H), 1.10–1.60 (m, 64H), 2.05–2.20 (m, 2H), 2.95–3.10 (m, 2H), 3.84 (d, J = 6.4 Hz, 2H), 4.25–4.40 (m, 1H), 7.60–7.75 (m, 1H), 7.80–7.95 (m, 1H). Anal. calcd for $C_{40}H_{79}N_2NaO_6S{\cdot}1.0H_2O$: C, 63.46; H, 10.78; N, 3.70; found: C, 63.43; H, 10.65; N, 3.79.

(*S*)-2-Propylcarbamoyl-2-(2-tetradecylhexadecanoylamino)-ethyl sulfate sodium salt (3j). Mp 135.0–138.0 °C (dec). ^{1}H NMR (DMSO-d_6) δ: 0.80–0.95 (m, 6H), 1.10–1.35 (m, 48H), 1.35–1.65 (m, 6H), 2.10–2.50 (m, 1H), 3.00–3.30 (m,2H), 4.05–4.40 (m, 2H), 4.65–4.85 (m, 1H), 6.90–7.30 (br, 2H). Anal. calcd for $C_{36}H_{71}N_2NaO_6S{\cdot}1.5H_2O$: C, 60.90; H, 10.50; N, 3.94; found: C, 60.52; H, 10.26; N, 3.67.

4-[(*S*)-2-Octadecanoylamino-2-(tetradecylcarbamoyl)-ethyl]phenyl sulfate sodium salt (2a). Chlorosulfonic acid (0.10 mL, 1.50 mmol) was added dropwise to pyridine (2 mL) at room temperature, and then the resulting mixture was stirred at 70–75 °C for 30 min. After the reaction mixture was cooled to room temperature, a solution of (*S*)-*N*-[2-(4-hydroxyphenyl)-1-(tetradecylcarbamoyl)ethyl] octadecanamide (0.33 g, mmol) in pyridine (5 mL) was added to the reaction mixture, and then the resulting mixture was stirred at 80–85 °C for 38 h. After evaporation of pyridine from the reaction mixture in vacuo, methanol (10 mL) was added to the obtained residue. A saturated aqueous solution of sodium carbonate was added to the resulting mixture till pH of the mixture showed 9. Water (10 mL) was added to the alkaline mixture, and then the precipitating solid was collected by filtration, and dried in vacuo. After the obtained solid was stirred with a mixture of chloroform and methanol (1:1, 50 mL), insoluble matter was removed by filtration. The obtained filtrate was concentrated in vacuo to dryness. Finally, the obtained residue was stirred with ethyl acetate (10 mL), and then the precipitating crystals were collected by filtration to give the title compound as a colorless powder (220 mg, Yield = 58%).

Mp 148.0 °C (dec). ^{1}H NMR (DMSO-d_6) δ: 0.80–0.95 (m, 6H), 1.10–1.50 (m, 54H), 1.95–2.10 (m, 2H), 2.60–3.10 (m, 4H), 4.30–4.50 (m, 1H), 6.95–7.15 (m, 4H), 7.80–8.00 (m, 2H). Anal. calcd for $C_{41}H_{73}N_2NaO_6S{\cdot}1.5H_2O$:C, 63.78; H, 9.92; N, 3.63; found: C, 63.49; H, 9.56; N, 3.61.

***N*-[(*S*)-4-Hydroxy-1-(tetradecylcarbamoyl)butyl]octadecanamide (29a).** Sodium borohydride (97 mg, 2.56 mmol) was added at room temperature to a solution of benzyl

(*S*)-4-(octadecanoylamino)-4-(tetradecylcarbamoyl)butanoate (1.00 g, 1.43 mmol) in THF (15 mL), and then the resulting mixture was heated to 70 °C. After addition of methanol (0.50 mL) to the mixture at the same temperature in 30 min, the reaction mixture was cooled to room temperature. After the solvent of the mixture had been evaporated in vacuo, the obtained residue was diluted with water (50 mL). The resulting mixture was extracted with chloroform, and then the chloroform layer was washed with a saturated solution of sodium chloride, dried over magnesium sulfate, and concentrated in vacuo to dryness. The obtained solid was purified by column chromatography (eluent chloroform: methanol = 20:1) to give the title compound as a colorless powder (140 mg, yield = 16%).

Mp 121.0–122.5 °C. ^{1}H NMR ($CDCl_3$) δ: 0.88 (t, J = 6.6 Hz, 6H), 1.15–1.40 (m, 50H), 1.40–2.00 (m, 8H), 2.20 (t, J = 7.6 Hz, 2H), 2.42 (br s, 1H), 3.10–3.35 (m, 2H), 3.60–3.80 (m, 2H), 4.40–4.60 (m, 1H), 6.37 (d, J = 8.1 Hz, 1H), 6.49 (br, 1H). Anal. calcd for $C_{37}H_{74}N_2O_3$: C, 74.69; H, 12.54; N, 4.71; found: C, 74.55; H, 12.51; N, 4.66.

(*S*)-4-Octadecanoylamino-4-(tetradecylcarbamoyl)butyl sulfate sodium salt (4a). The title compound was synthesized from the hydroxyl derivative (**29a**) in the same manner as **3a**.

Mp 164.0–166.0 °C (dec). ^{1}H NMR (DMSO-d_6) δ: 0.84 (t, J = 6.5 Hz, 6H), 1.10–1.80 (m, 58H), 2.09 (t, J = 7.3 Hz, 2H), 2.90–3.10 (m, 2H), 3.65–3.80 (m, 2H), 4.10–4.25 (m, 1H), 7.60–7.80 (m, 2H). Anal. calcd for $C_{37}H_{73}N_2NaO_6S{\cdot}1.0H_2O$: C, 62.15; H, 10.57; N, 3.92; found: C, 62.29; H, 10.39; N, 3.90.

(*S*)-4-Tetradecylcarbamoyl-4-octadecanoylaminobutanoic acid (6a). Benzyl (*S*)-4-tetradecylcarbamoyl-4-octadecanoylaminobutanoate (**27a**) (1.00 g, 1.43 mmol) was dissolved in a mixture of methanol (5 mL) and dioxane (20 mL) with heating. A mixture of Pd/C (10%, 1.52 g) and dioxane (10 mL) was added to the above solution, and then resulting mixture was stirred at room temperature under hydrogen atmosphere for 2 h. Pd–C was removed by filtration to give a filtrate. The obtained Pd–C was washed with chloroform, and the obtained wash was combined with the filtrate. The resulting solution was concentrated in vacuo to afford the title compound as a colorless powder (750 mg, Yield = 86%). Recrystallization of the obtained powder from ethyl acetate gave an analytical sample.

Mp 112.5–115.5 °C. ^{1}H NMR (DMSO-d_6) δ: 0.75–0.95 (m, 6H), 1.10–1.60 (m, 54H), 1.60–2.00 (m, 2H), 2.10 (t, J = 7.3 Hz, 2H), 2.18 (t, J = 7.4 Hz, 2H), 2.90–3.15 (m, 2H), 4.10–4.30 (m, 1H), 7.65–7.90 (m, 2H), 12.01 (br s, 1H). Anal. calcd for $C_{37}H_{72}N_2O_4$: C, 72.97; H, 11.92; N, 4.60; found: C, 72.81; H, 11.91; N, 4.54.

***N*-[(*S*)-3-Methanesulfonyl-1-(tetradecylcarbamoyl)propyl]octadecanamide (11a).** A solution of *m*-chloroperbenzoic acid (*m*CPBA, 70%, 43 mg, 0.175 mmol) in dichloromethane (5 mL) was added at room temperature to a solution of *N*-[(*S*)-3-methanesulfinyl-1-(tetradecylcarbamoyl)propyl]octadecanamide (**10a**) (100 mg, 0.159 mmol) in dichloromethane (5 mL), and then the resulting mixture was stirred at room temperature for 16 h. After addition of a saturated aqueous solution of sodium hydrogen carbonate (15 mL), the resulting mixture was stirred at room temperature for 5 min. Chloroform (20 mL) was added to the mixture, and then the organic layer was separated. The aqueous layer was extracted with chloroform twice, and then the organic layers were combined. The obtained solution was washed with a saturated aqueous solution of sodium chloride, dried over magnesium sulfate, and concentrated in vacuo. The obtained colorless powder was recrystallized from ethyl acetate to yield the target compound as a colorless powder (21.8 mg, Yield = 21%).

Mp 130.0–132.0 °C. ^{1}H NMR ($CDCl_3$) δ: 0.88 (t, J = 6.6 Hz, 6H), 1.15–1.40 (m, 50H), 1.40–1.70 (m, 4H), 2.10–2.35 (m, 4H), 2.99 (s, 3H), 2.90–3.40 (m, 4H), 4.61 (dd, J = 6.8, 6.8 Hz, 1H), 6.30–6.50 (m, 2H). Anal. calcd for $C_{37}H_{74}N_2O_4S{\cdot}0.5H_2O$: C, 68.15; H, 11.59; N, 4.29; found: C, 68.07; H, 11.24; N, 4.10.

***N*-[(*R*)-2-Mercapto-1-(tetradecylcarbamoyl)ethyl]octadecanamide (9a).** A mixture of (**30a**) (333 mg, 0.286 mmol), tributylphosphine (116 mg, 0.572 mmol), methanol (20 mL), and water (0.5 mL) was stirred at room temperature for 19 h. Chloroform (100 mL) was added to the reaction mixture, and then the resulting mixture was washed with water, and then dried over magnesium sulfate. The solution was concentrated in vacuo, and then the obtained colorless oil was purified by column chromatography (eluent first, chloroform, then chloroform/methanol = 20:1) to give the title compound as a colorless powder (168 mg, Yield = 50%).

^{1}H NMR ($CDCl_3$) δ: 0.88 (t, J = 6.6 Hz, 6H), 1.20–1.40 (m, 50H), 1.40–1.75 (m, 4H), 1.67 (dd, J = 7.7, 10.7 Hz, 1H), 2.25 (t, J = 7.5 Hz, 2H), 2.70 (ddd, J = 7.0, 10.1, 13.8 Hz, 1H), 3.04 (ddd, J = 4.3, 7.7, 13.8 Hz, 1H), 3.20–3.40 (m, 2H), 4.52 (ddd, J = 4.3, 7.0, 8.1 Hz, 1H), 6.30 (br, 1H), 6.39 (d, J = 8.1 Hz, 1H). Anal. calcd for $C_{35}H_{70}N_2O_2S$: C, 72.10; H, 12.10; N, 4.80; found: C, 72.11; H, 12.38; N, 5.01.

***N*-[(*R*)-2-(3-Hydroxypropylthio)-1-(tetradecylcarbamoyl)ethyl]octadecanamide (31a).** Potassium carbonate (238 mg, 1.72 mmol) was added to a solution of *N*-[(*R*)-2-mercapto-1-(tetradecylcarbamoyl)ethyl]octadecanamide (**9a**) (500 mg, 0.858 mmol) and 3-bromopropanol (144 mg, 1.03 mmol) in DMF (6 mL) and THF (3 mL), and then the resulting mixture was stirred at room temperature for 64 h. After addition of water (100 mL) to the reaction mixture, the diluted mixture was extracted with ethyl acetate. The organic layer was washed with water, and dried over magnesium sulfate. The solution was concentrated in vacuo, and finally the obtained residue was purified by column chromatography (eluent *n*-hexane/ethyl acetate = 1:1) to give the title compound as a colorless powder (237 mg, yield = 50%).

^{1}H NMR ($CDCl_3$) δ: 0.88 (t, J = 6.6 Hz, 6H), 1.10–1.40 (m, 50H), 1.40–1.80 (m, 5H), 1.80–1.95 (m, 2H), 2.22 (t, J = 7.5 Hz, 2H), 2.60–3.05 (m, 4H), 3.15–3.35 (m, 2H), 3.65–3.80 (m, 2H), 4.40–4.55 (m, 1H), 6.40–6.55 (m, 2H). Anal. calcd for $C_{38}H_{76}N_2O_3S$: C, 71.19; H, 11.95; N, 4.37; found: C, 71.20; H, 11.96; N, 4.66.

3-[(*R*)-2-Octadecanoylamino-2-(tetradecylcarbamoyl)-ethyl]thiopropyl sulfate sodium salt (5a). The title compound was synthesized from **31a** in the same manner as **3a**.

Mp 183.0–185.0 °C (dec). ^{1}H NMR ($CDCl_3$) δ: 0.88 (t, J = 6.6 Hz, 6H), 1.20–1.50 (m, 50H), 1.50–1.80 (m, 4H), 1.95–2.15 (m, 2H), 2.21 (t, J = 7.5 Hz, 2H), 2.65–3.10 (m, 4H), 3.15–3.45 (m, 2H), 4.15–4.35 (m, 2H), 4.60–4.80 (m, 1H), 6.85–7.00 (m, 1H), 7.30–7.45 (m, 1H). Anal. calcd for $C_{38}H_{75}N_2NaO_6S_2{\cdot}1.0H_2O$: C, 59.96; H, 10.20; N, 3.68; found: C, 59.66; H, 9.99; N, 3.97.

***N*-(2-Hydroxyethyl)-2-tetradecylhexadecanamide (33j).** The title compound was synthesized from 2-aminoethanol and 2-tetradecylhexadecanoyl chloride in the same manner as compound **26**.

Mp 116.0–117.5 °C. ^{1}H NMR ($CDCl_3$) δ: 0.88 (t, J = 6.6 Hz, 6H), 1.10–1.70 (m, 52H), 1.95–2.10 (m, 1H), 2.59 (br s, 1H), 3.35–3.50 (m, 2H), 3.65–3.80 (m, 2H), 5.75–5.90 (m, 1H). Anal. calcd for $C_{32}H_{65}NO_2$: C, 77.51; H, 13.21; N, 2.82; found: C, 77.56; H, 13.17; N, 2.69.

2-(2-Tetradecylhexadecanoyl)aminoethyl sulfate sodium salt (12j). The title compound was synthesized from **33j** in the same manner as compound **3a**. Mp 125.0–126.0 °C. ^{1}H NMR ($CDCl_3$) δ: 0.88 (t, J = 6.5 Hz, 6H), 1.10–1.70 (m, 52H), 2.00–2.20 (m, 1H), 3.40–3.70 (m, 2H), 4.00–4.25 (m, 2H), 6.93 (br s, 1H). Anal. calcd for $C_{32}H_{64}NNaO_5S{\cdot}1.0H_2O$: C, 62.40; H, 10.80; N, 2.27; found: C, 62.47; H, 10.50; N, 2.21.

***N*-(2-Hydroxy-3-octadecanoylaminopropyl)octadecanamide (35q).** A solution of octadecanoyl chloride (2.76 g, 9.10 mmol) in THF (30 mL) was added dropwise to a mixture of 1,3-diamino-2-propanol (**34**) (400 mg, 4.44 mmol), triethylamine (990 mg, 9.77 mmol), and THF (200 mL) under ice-cooling, and then the resulting mixture was stirred at room temperature for 20 h. After the reaction mixture had been diluted with water (100 mL) to the reaction mixture, the resulting mixture was extracted with chloroform. Combined organic layer was washed with a diluted hydrochloric acid, a saturated aqueous solution of sodium hydrogen carbonate, and a saturated aqueous solution of sodium chloride succesively, dried over magnesium sulfate, and concentrated in vacuo. The obtained solid was recrystallized from ethanol to give the title compound as a colorless crystalline powder (2.25 g, Yield = 81%). Recrystallization from ethyl acetate gave an analytical sample having the following physical chemical properties.

Mp 127.0–128.5 °C. ^{1}H NMR ($CDCl_3$) δ: 0.88 (t, J = 6.5 Hz, 6H), 1.15–1.40 (m, 56H), 1.40–1.75 (m, 4H), 2.22 (t, J = 7.6 Hz, 4H), 3.15–3.50 (m, 4H), 3.70–3.85 (m, 1H), 3.95–4.10 (br, 1H), 6.15–6.35 (br, 2H). Anal. calcd for $C_{39}H_{78}N_2O_3$: C, 75.18; H, 12.62; N, 4.50; found: C, 74.87; H, 12.53; N, 4.63.

2-Octadecanoylamino-1-(octadecanoylaminomethyl)ethyl sulfate sodium salt (13q). The title compound was synthesized from **35q** in the same manner as compound **3a**.

Mp 144.0–147.0 °C (dec). ^{1}H NMR ($CDCl_3$) δ: 0.88 (t, J = 6.6 Hz, 6H), 1.15–1.40 (m, 56H), 1.40–1.70 (m, 4H), 2.19 (t, J = 7.5 Hz, 4H), 3.15–3.40 (m, 2H), 3.40–3.65 (m, 2H), 4.35–4.50 (m, 1H), 7.10–7.50 (br, 2H). Anal. calcd for $C_{39}H_{77}N_2NaO_6S{\cdot}1.25H_2O$: C, 62.66; H, 10.72; N, 3.75; found: C, 62.62; H, 10.59; N, 3.47.

The following compounds **13r** and **13s** were synthesized from 1,3-diamino-2-propanol and the corresponding acyl chloride via the corresponding hydroxyl derivative in the same manner as compound **13q**.

2-(3-Tetradecyloxypropanoyl)amino-1-[(3-tetradecyloxypropanoyl)amino methyl]ethyl sulfate sodium salt (13r). Mp 143.5–146.0 °C (dec). ^{1}H NMR ($CDCl_3$) δ: 0.88 (t, J = 6.6 Hz, 6H), 1.10–1.45 (m, 44H), 1.45–1.65 (m, 4H), 2.35–2.60 (m, 4H), 3.20–3.80 (m, 12H), 4.40–4.55 (m, 1H), 7.30–7.50 (br, 2H). Anal. calcd for $C_{37}H_{73}N_2NaO_8S{\cdot}2.0H_2O$: C, 58.09; H, 10.14; N, 3.66; found: C, 57.88; H, 9.83; N, 3.60.

2-(15-Ethoxypentadecanoyl)amino-1-[(15-ethoxypentadecanoyl)aminomethyl] ethyl sulfate sodium salt (13s). Mp 110.0–112.0 °C (dec). ^{1}H NMR ($CDCl_3$) δ: 1.19 (t, J = 7.0 Hz, 6H), 1.00–1.45 (m, 40H), 1.45–1.75 (m, 8H), 2.05–2.35 (m, 4H), 3.15–3.70 (m, 4H), 3.39 (t, J = 6.6 Hz, 4H), 3.46 (q, J = 7.0 Hz, 4H), 4.35–4.55 (m, 1H), 7.10–7.45 (br, 2H). Anal. calcd for $C_{37}H_{73}N_2NaO_8S{\cdot}1.3H_2O$: C, 59.06; H, 10.13; N, 3.72; found: C, 59.09; H, 9.89; N, 3.67.

Inhibitory activity against incorporation of DiI-acetyl-LDL into macrophages

Dulbecco's modified Eagle medium (DMEM) and fetal calf serum (FCS) were purchased from GIBCO BRL (Grand Island, NY, USA). Lipoprotein-deficient serum (LPDS) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Na^{125}I was purchased from Du Pont New England Nuclear (Boston, MA, USA). Female ddY mice were obtained from Nihon SLC Co. (Shizuoka, Japan). BCA Protein Assay Reagent Kit was purchased from PIERCE (Rockford, IL, USA). All other reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

LDL was isolated from normolipidemic human plasma containing 0.1% EDTA, 0.02% sodium azide and 0.5 mg/mL benzamidine at a density of 1.019–1.063 g/mL by sequential ultracentrifugation.[24] LDL was acetylated by repeated additions of acetic anhydride as described.[25] DiI-acetyl-LDL was prepared by co-incubation of acetyl-LDL with DiI solution as described.[26] Acetyl-LDL was radioiodinated at a specific activity of

100–300 cpm/ng protein with $Na^{125}I$ using the iodine monochloride method.[27]

Mouse peritoneal macrophages were prepared according to the method of Cohn and Morse.[28] Peritoneal macrophages were harvested in phosphate-buffered saline without Ca^{2+} and Mg^{2+} (PBS[−]) from non-stimulated female ddY mice (25–30 g). The cells were centrifuged at 800*g* for 5 min, washed once with PBS[−], and suspended at 2×10^6 cells/mL in DMEM containing 10% FCS, 100 U/mL penicillin and 100 μg/mL streptomycin. The cells suspension (0.4 mL) was plated in 24-well plastic dishes for uptake assay, and the suspension (1 mL) was plated in 12-well plastic dishes for binding assay. After an incubation at 37 °C for 2 h, non-adherent cells were removed by washing twice with PBS[−]. Adherent cell monolayers were cultured with 1 mL of DMEM containing 10% FCS, and were used for the experiments next day.

The cells were incubated for 4 h at 37 °C in 0.3 mL of DMEM containing 10% LPDS, 10 μg/mL DiI-Ac-LDL, and a drug. After the incubation, the cells were washed once rapidly and twice with ice-cold 50 mM Tris–HCl (pH 7.4), 150 mM NaCl, 2 mg/mL BSA, and were washed once with ice-cold 50 mM Tris–HCl (pH 7.4), 150 mM NaCl. The cells were dissolved in 2 mM SDS, and the fluorescence was measured at the excitation wavelength of 530 nm and at the emission wavelength of 590 nm. The concentration of cell protein was determined by BCA Protein Assay Reagent Kit.

^{125}I-Acetyl-LDL binding assay

The cells were pre-incubated in DMEM containing 10 mM HEPES-Na (pH 7.4) and 10% LPDS for 30 min at 4 °C. The medium was then changed to 1 mL of DMEM containing 10 mM HEPES-Na (pH 7.4), 10% LPDS, 10 μg/mL ^{125}I-Ac-LDL, and a drug, and the cell were further incubated for 2 h at 4 °C. Non-specific binding was estimated in the presence of a 40-fold excess unlabeled Ac-LDL. After the incubation, the cells were washed once rapidly and twice for 10 min with ice-cold 50 mM Tris–HCl (pH 7.4), 150 mM NaCl, 2 mg/mL BSA, and were washed once with ice-cold 50 mM Tris–HCl(pH 7.4), 150 mM NaCl. The cells were dissolved in 0.1 N NaOH, and the amount of ^{125}I-Ac-LDL bound to cells was counted in a gamma counter. The concentration of cell protein was determined by the method of Lowry et al.[29]

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