Stereoselective Synthesis and Structure−Activity Relationship of Novel Ceramide Trafficking Inhibitors. (1*R*,3*R*)-*N*-(3-Hydroxy-1-hydroxymethyl-3-phenylpropyl)dodecanamide and Its Analogues

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New ceramide trafficking inhibitors, (1*R*,3*R*)-*N*-(3-hydroxy-1-hydroxymethyl-3-phenylpropyl)-dodecanamide (HPA-12) and a series of its analogues, were synthesized in diastereomerically and enantiomerically pure forms, and the structure−activity relationship was investigated. These analogues were stereoselectively synthesized via catalytic enantioselective Mannich-type reactions using a Cu(II)−chiral diamine **4** complex. Analysis of HPA-12 analogues having various lengths of the amide side chain showed that the optimal chain length for the inhibition of sphingomyelin biosynthesis is 13 with an IC_{50} of ∼50 nM. Masking of the hydroxy group at the 2′- or 3-position of HPA-12 was carried out by methylation, and it was revealed that these hydroxy groups were essential for the activity. Installation of another hydroxy group onto HPA-12 at the same position as that in the natural ceramide was also conducted, but no enhancement of the activity was observed.

Introduction

Sphingolipids are ubiquitous constituents of the plasma membrane in eukaryotic cells. Sphingolipids play important roles in various biological events including cell proliferation, differentiation, and apotosis, etc.[1−4] Thus, inhibitors of sphingolipid biosynthesis are useful tools for investigating the functions of sphingolipids and for creating new types of drugs.[1a] Our group has also focused on inhibitors such as sphingofungin B and F,[5] khafrefungine,[6] etc., and the total synthesis and clarification of the structural relationship of the biological activity of these inhibitors have been accomplished.

In mammalian cells, sphingolipid biosynthesis is initiated by the condensation of serine and palmitoyl coenzyme A to generate ketodihydrosphingosine, which is, after three different enzymatic steps, converted to ceramide in the endoplasmic reticulum (ER).[7] Ceramide is converted to sphingomyelin (SM) or glycosphingolipids in the Golgi apparatus.[7] The main pathway of ceramide transport from the ER to the site of SM biosynthesis has been shown to be an ATP- and cytosol-dependent pathway, which is different from the ER-to-Golgi transport pathway of glycoproteins.[8,9] In our search for novel molecules that influence sphingolipid biosynthesis, we have found that (1*R*,3*R*)-*N*-(3-hydroxy-1-hydroxymethyl-3-phenylpropyl)dodecanamide (HPA-12 (**1**), Figure 1) inhibits the main pathway of ceramide transport from the ER to the site of SM synthesis, thereby inhibiting de novo biosynthesis of SM.[10] We also showed that the (1*R*,3*R*)-configuration of HPA-12 is the most active among the four stereoisomers.[10] In addition, we have established an efficient route for enantioselective synthesis of (1*R*,3*R*)-HPA-12 by using a chiral Cu(II)−diamine complex as a chiral catalyst.[11−13]

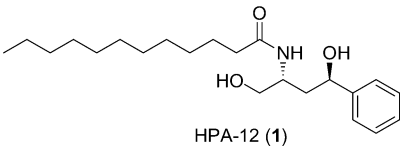

Figure 1. Novel inhibitor of ceramide trafficking, HPA-12.

In the present work, we synthesized several HPA-12 analogues in diastereomerically and enantiomerically pure forms by the chiral Cu(II)−diamine catalyzed Mannich-type reactions. Bioassays with the HPA-12 analogues determined the optimal length of the amide side chain of the drug and showed the vital roles of the hydroxy groups of HPA-12 for the bioactivity.

Results and Discussion

We have recently developed an efficient synthetic method for HPA-12 using chiral Cu(II)-catalyzed asymmetric Mannich-type reactions of *N*-acylimino esters.[12] In the present study, we applied this methodology to the synthesis of various HPA analogues.

First, we prepared HPA analogues that have amide side chains of various lengths (HPA-10 to -18). Asymmetric Mannich-type reactions of α-*N*-acylimino esters **2a**−**d** proceeded in high yields with high enantioselectivities using a Cu(II)−chiral diamine **4** complex (Scheme 1). The Mannich adducts **5a**−**d** were reduced by a combination of K-selectride and $LiBEt_3H$ to afford HPA-11 to -14 (**6a**−**d**) with high *anti* selectivities (*syn*/*anti*

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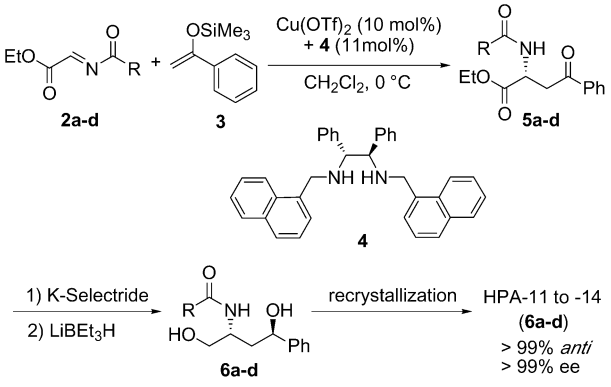
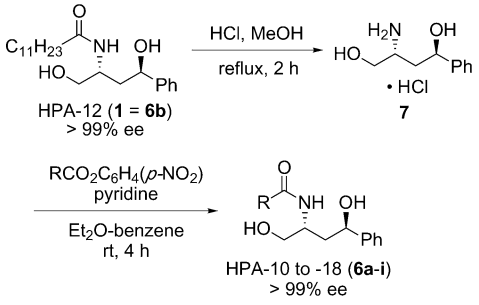
Scheme 1. Synthesis of HPA Analogues Using Cu(II)−Diamine Catalyzed Mannich-Type Reactions



Imino ester	5a-d Yield (%)	5a-d Ee (%)	6a-d Yield (%)	6a-d *Syn*/*Anti*	Product
2a: R = $C_{10}H_{21}$	88	94	87[a]	16/84	HPA-11 (**6a**)
2b: R = $C_{11}H_{23}$	92	94	99[b]	9/91	HPA-12 (**6b** = **1**)
2c: R = $C_{12}H_{25}$	91	92	93[a]	16/84	HPA-13 (**6c**)
2d: R = $C_{13}H_{27}$	91	92	88[a]	16/84	HPA-14 (**6d**)

[a] Reaction was performed in 1,2-dimethoxyethane at -45 °C. [b] Reaction was performed in tetrahydrofuran at -78 °C.

Scheme 2. Synthesis of HPA Analogues by the Amide Exchange of HPA-12



R	Yield (%)
C_9H_{19} (HPA-10, **6e**)	83
$C_{10}H_{21}$ (HPA-11, **6a**)	81
$C_{12}H_{25}$ (HPA-13, **6c**)	75
$C_{13}H_{27}$ (HPA-14, **6d**)	72
$C_{14}H_{29}$ (HPA-15, **6f**)	55
$C_{15}H_{31}$ (HPA-16, **6g**)	61
$C_{16}H_{33}$ (HPA-17, **6h**)	68
$C_{17}H_{35}$ (HPA-18, **6i**)	51

= 16/84 to 9/91) in excellent yields.[12] In the reduction step, a very small amount of moisture was found to decrease the diastereoselectivity (*syn*/*anti* ≈ 4/6) because of partial epimerization of the *anti* isomer to the syn isomer. To avoid this epimerization, the solvents THF and 1,2-dimethoxyethane were distilled from ketyl before use. Finally, these HPA analogues **6a**−**d** were easily purified by recrystallization from dichloromethane/*n*-hexane to give diastereomerically and enantiomerically pure HPA-11 to -14.

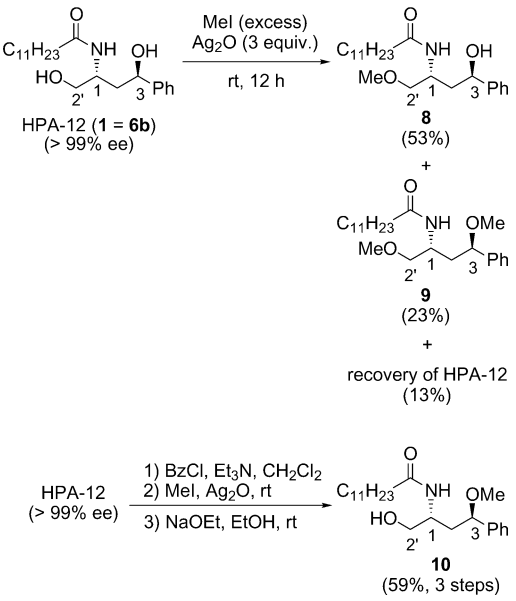
These HPA analogues were also prepared by amide exchange of HPA-12 (Scheme 2). The *N*-acyl group of HPA-12 was removed by refluxing with hydrogen chloride in methanol, and then acyl groups of various lengths were reintroduced selectively on the nitrogen by *p*-nitrophenol esters.[14] It is noted that no epimerization occurred during these transformations.

Table 1. Effect of HPA-12 (**1**) and Its Analogues Having Various Chain Lengths (**6a**−**i**) on de Novo Synthesis of SM in CHO Cells

compd	IC_{50} [a] (nM)	compd	IC_{50} [a] (nM)
C_9H_{19} (HPA-10, **6e**)	300	$C_{14}H_{29}$ (HPA-15, **6f**)	90
$C_{10}H_{21}$ (HPA-11, **6a**)	110	$C_{15}H_{31}$ (HPA-16, **6g**)	>1000
$C_{11}H_{23}$ (HPA-12, **6b** = **1**)	70	$C_{16}H_{33}$ (HPA-17, **6h**)	>1000
$C_{12}H_{25}$ (HPA-13, **6c**)	50	$C_{17}H_{35}$ (HPA-18, **6i**)	>1000
$C_{13}H_{27}$ (HPA-14, **6d**)	70		

[a] The effect of the compounds at various concentrations (0 to 1 μM) on *de novo* synthesis of SM was examined by metabolic labeling of lipids in CHO cells with radioactive serine. The IC_{50} values were determined by *Scatchard* plot analysis of the data obtained from a single series of assays for each compound.

Scheme 3. Selective Methylation of Hydroxy Groups of HPA-12



We previously demonstrated that HPA-12 inhibits intracellular ceramide transport, but not the enzyme activity of SM synthase, thereby inhibiting SM biosynthesis in intact mammalian cells.[10] To determine the activity of the HPA-12 analogues as inhibitors of ceramide transport, we examined the effect of these compounds on SM biosynthesis by metabolic labeling of lipids in Chinese hamster ovary (CHO) cells with radioactive L-serine. Among these analogues, HPA-13 (**6c**) most effectively inhibited SM biosynthesis with ∼50 nM, causing 50% inhibition (IC_{50}, Table 1). The chain lengths of 11, 12, 14, and 15 (**6a**, **6b** (=**1**), **6d**, **6f**, respectively) were similarly effective with their IC_{50} of 70−110 nM, while other chain lengths (**6e**,**g**−**i**) were ineffective or far less effective (Table 1). Thus, middle chain lengths (C11−C15) were shown to be important for the potent bioactivity of HPA compounds.

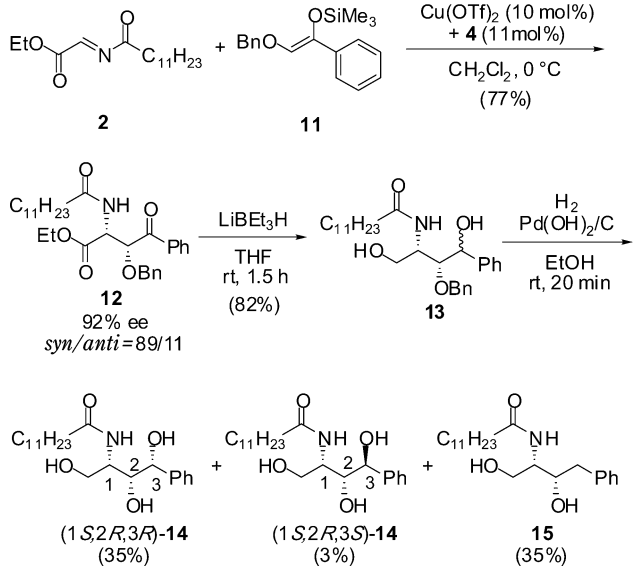
To investigate the effect of the hydroxy groups of HPA-12 on the bioactivity, we synthesized various methylated forms of HPA-12 (Scheme 3). 2′-Methoxy- and 2′,3-dimethoxy-HPA-12 (**8** and **9**) were prepared by direct methylation of HPA-12 with MeI and Ag_2O. Selective methylation at the 3-hydroxy group was carried out via benzoyl protection/deprotection at the 2′-position to give 3-methoxy-HPA-12 (**10**) in good yield.[15]

Table 2. Effect of **1** (=**6b**) and **8**−**10** on de Novo Synthesis of SM in CHO Cells

compd	SM biosynthesis[a] (% of vehicle control)
none (vehicle control)	100 ± 23
1 = **6b**	4.0 ± 0.3
8	79 ± 4
9	130 ± 6
10	119 ± 9

[a] The effect of the compounds at 1 μM on de novo synthesis of SM was examined by metabolic labeling of lipids in CHO cells with radioactive serine. The data (mean ± SD, $n = 3$) are represented as the percentage of the mean value of the vehicle control, in which cells were treated with 0.01% (v/v) DMSO in the absence of the HPA derivatives.

Scheme 4. Synthesis of 2-Hydroxy Analogues of HPA-12 Using Cu(II)−Diamine Catalyzed Mannich-Type Reactions



Bioassays showed that none of these methoxy forms are able to inhibit SM biosynthesis in intact cells even at 1 μM (Table 2). Therefore, both the 2′- and 3- hydroxy groups of HPA-12 are essential for the potent activity of HPA-12.

We also synthesized the 2-hydroxy-installed HPA analogue **14** because natural ceramide has a hydroxy group at the corresponding position. Four diastereomers of **14** were prepared stereoselectively as follows. (1*S*,2*R*,3*R*)-**14** was synthesized using the Cu(OTf)$_2$−diamine catalyzed Mannich-type reaction of **2b** with 2-benzyloxy-1-phenyl-1-trimethylsiloxyethene (**11**) (Scheme 4). The Mannich-type reaction proceeded with high syn selectivity (*syn*/*anti* = 89:11) and high enantioselectivity (*syn* isomer: 92% ee), followed by reduction and debenzylation to give the desired (1*S*,2*R*,3*R*)-**14**. While hydrogenolysis of the benzyloxy group of **13** using Pd/C was found to be very sluggish, Pd(OH)$_2$/C gave the desired triol in moderate yield along with overreduction product **15**. The other three isomers of **14** were synthesized from L-serine (Scheme 5). Diol **16** was prepared from L-serine according to the literature procedure,[16] and then both the Boc and acetonide groups were removed under acidic conditions followed by N-acylation to give (1*S*,2*S*,3*S*)-**14**. (1*S*,2*R*,3*S*)- and (1*S*,2*S*,3*R*)-**14** were also synthesized in a similar way from L-serine-derived **17**.[16] Dihydroxylation of **17** was conveniently carried out using microencapsulated osmium tetraoxide[17] to give triols, **18** and **19**, in good yields. These diastereomers, **18** and **19**, were separated by chromatography on silica gel, and then they were converted to the desired (1*S*,2*R*,3*S*)- and (1*S*,2*S*,3*R*)-**14** in the same manner as described above.

Scheme 5. Synthesis of 2-Hydroxy Analogues of HPA-12 from L-Serine

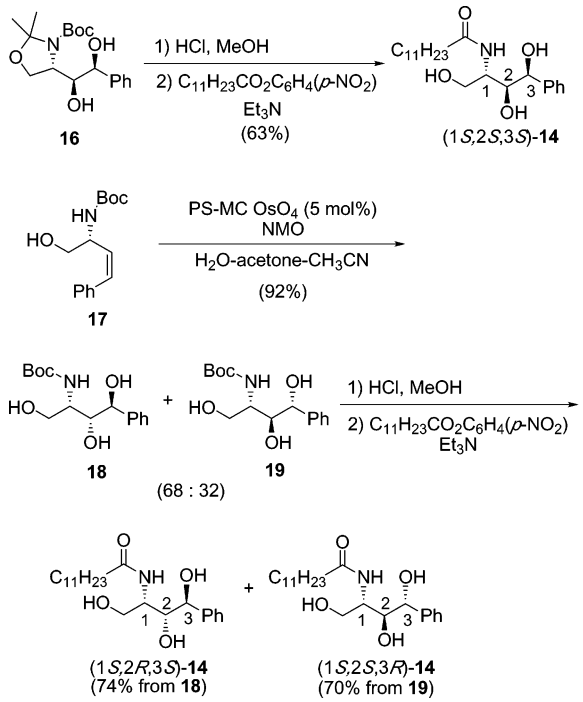


Table 3. Effect of **1** (=**6b**), (1*S*,2*R*,3*R*)-**14**, (1*S*,2*S*,3*S*)-**14**, (1*S*,2*R*,3*S*)-**14**, and (1*S*,2*S*,3*R*)-**14** on de Novo Synthesis of SM in CHO Cells

compd	SM biosynthesis[a] (% of vehicle control)
none (vehicle control)	100 ± 10
1 = **6b**	8.2 ± 1.0
(1*S*,2*R*,3*R*)-**14**	40 ± 3
(1*S*,2*S*,3*S*)-**14**	28 ± 2
(1*S*,2*R*,3*S*)-**14**	57 ± 2
(1*S*,2*S*,3*R*)-**14**	17 ± 1

[a] The effect of the compounds at 1 μM on de novo synthesis of SM was examined by metabolic labeling of lipids in CHO cells with radioactive serine. The data (mean ± SD, $n = 3$) are represented as the percentage of the mean value of the vehicle control, in which cells were treated with 0.01% (v/v) DMSO in the absence of the HPA derivatives.

The (1*R*,3*R*)-configuration of HPA-12 is the most active among the four stereoisomers, and this configuration of HPA-12 resembles the stereochemistry of natural ceramide.[10] On the basis of these results, we hypothesized that HPA-12 interacts antagonistically with a ceramide-recognition factor involved in intracellular ceramide trafficking, although the factor remains unidentified.[10] As shown in the present study, HPA having a C10 chain is less effective than HPAs having C11−C15 chains. In addition, HPA having a C8 or C3 chain is far less effective or is ineffective compared to

HPA-12, as shown previously.[10] Thus, shortening of the amide acyl chain below C11 may considerably weaken the interaction of HPA derivatives with the hypothetical factor targeted by HPA-12. Although natural ceramide has C16 or longer acyl chains, HPA derivatives having such long chains are ineffective as inhibitors of SM biosynthesis. The aqueous insolubility of these HPA derivatives might prevent them from accessing the cytoplasm of cells, and this nature could be ameliorated by using a middle chain length (C11−C15) as the amide acyl chain. The requirement of both 2′- and 3-hydroxy groups of HPA12 for its bioactivity might also indicate that both of the hydroxyl groups are important for the interaction of HPA-12 with its targeting factor.

Conclusion

HPA-12 is an inhibitor of intracellular transport of ceramide from the ER to the Golgi compartment for SM biosynthesis.[10] In the present study, we synthesized a series of HPA-12 analogues via catalytic asymmetric Mannich-type reactions using a Cu(II)−chiral diamine **4** complex. This methodology provides an efficient and concise approach to a series of HPA analogues in a diastereoselective and enantioselective manner. Bioassays with these analogues showed that the optimal length of the amide side chain is 13 and that the hydroxy groups at the 2′- and 3-position are essential for the inhibition of SM biosynthesis. Installation of another hydroxy group at the position where natural ceramide has a hydroxy group did not enhance the bioactivity. Collectively, we conclude that the structure of (1*R*,3*R*)-HPA-12 is almost optimal among phenylpropyl derivatives in terms of the inhibition of intracellular ceramide trafficking.

Experimental Section

General. Melting points are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM-LA300, JNM-LA400, or JNM-LA500 spectrometer. Tetramethylsilane (TMS) served as an internal standard (δ 0) for ^{1}H NMR, and $CDCl_3$ was used as an internal standard (δ 77.0) for ^{13}C NMR. IR spectra were measured with a JASCO FT/IR-610 spectrometer. Optical rotations were measured with a JASCO P-1010 polarimeter. Mass spectrometry analysis was carried out with a JEOL JMS-700. HPLC analyses were carried out using the following apparatuses: Shimadzu LC-10AT (liquid chromatograph), Shimadzu SPD-10A (UV detector), and Shimadzu C-R6A or C-R8A Chromatopac. Elemental analyses were performed on a Yanaco CHN Corder MT-6. Column chromatography was conducted on silica gel 60 (Merck), and preparative thin-layer chromatography was carried out using Wakogel B-5F. All reactions were carried out under an argon atmosphere in dried glassware unless otherwise indicated. All reaction solvents were dried or distilled by standard procedures.

Preparation of α-Imino Esters (2a−d). To a suspension of α-hydroxyglycine ethyl ester (13.3 mmol) in CH_2Cl_2 (40 mL) was added thionyl chloride (9.7 mL, 133 mmol). The reaction mixture was refluxed for 1 h and cooled to room temperature (rt). The solvent was removed in vacuo to give the almost pure α-chloro glycine ester in quantitative yield. Further purification was done by washing the white precipitate with petroleum ether under argon atmosphere three times. To a mixture of this product (0.45 mmol) and 3.5 mmol/g piperidinomethylpolystyrene (257 mg, 0.90 mmol) was added CH_2Cl_2 (4.5 mL). The reaction mixture was stirred at room temperature for 10 min, and then the stirring was stopped. The polymer floated on the surface, and the clear part of the solution (2.5 mL, 0.25 mmol of imine **2a**−**d**) was taken with a gastight syringe. This solution was used immediately in the following Mannich-type reactions.

General Procedure for Mannich-Type Reactions Using a Chiral Copper Catalyst Prepared from Cu(OTf)$_2$ and Chiral Diamine Ligand 4. $Cu(OTf)_2$ (9.0 mg, 0.025 mmol) was dried for 2 h under vacuum at 100 °C. Ligand **4** (13.5 mg, 0.028 mmol) was added to the $Cu(OTf)_2$ flask under an argon atmosphere, and then CH_2Cl_2 (1.0 mL) was added. The light-blue solution was stirred for over 2 h until the color changed to dark-green. CH_2Cl_2 (3.2 mL) was added, and then the solution was cooled to 0 °C. Silyl enol ether **3** (0.375 mmol) in CH_2Cl_2 (0.8 mL) was added to this mixture. *N*-Acylimino ester **2** (0.25 mmol) in CH_2Cl_2 (2.5 mL) was then added slowly over 20 min, and the reaction mixture was further stirred at 0 °C for 12 h. The reaction was quenched by addition of saturated aqueous $NaHCO_3$, and the reaction mixture was allowed to warm to room temperature and stirred until the color of the aqueous layer changed to blue. The mixture was extracted with CH_2Cl_2. The organic layer was washed with brine and dried over anhydrous Mg_2SO_4, and the solvent was evaporated. The residue was dissolved in CH_2Cl_2 (3.0 mL), and 4 N HCl in AcOEt (0.1 mL) was added to the solution. The mixture was stirred at room temperature for 10 min and then evaporated to dryness. The crude product was purified by chromatography on silica gel to afford the desired Mannich adduct.

(2*R*)-4-Oxo-4-phenyl-2-undecanoylaminobutyric acid ethyl ester (5a): $[\alpha]\ ^{29}_D$ −58.7 (94% ee, *c* 1.00, $CHCl_3$); mp 41−42 °C; ^{1}H NMR ($CDCl_3$) δ 0.87 (t, 3H, J = 6.8 Hz), 1.15−1.35 (m, 17H), 1.55−1.70 (m, 2H), 2.20 (dt, 2H, J = 3.3, 7.6 Hz), 3.59 (dd, 1H, J = 4.1, 18.3 Hz), 3.75 (dd, 1H, J = 4.0, 18.3 Hz), 4.21 (q, 2H, J = 7.1 Hz), 4.96 (dt, 1H, J = 8.0, 3.9 Hz), 6.60 (brd, 1H, J = 8.0 Hz), 7.48 (apparent t, 2H, J = 7.7 Hz), 7.60 (apparent t, 1H, J = 7.4 Hz), 7.94 (apparent d, 2H, J = 7.3 Hz); ^{13}C NMR ($CDCl_3$) δ 14.0, 14.1, 22.6, 25.5, 29.1, 29.2, 29.3, 29.4, 29.5, 31.8, 36.5, 40.5, 48.2, 61.7, 128.1, 128.7, 133.7, 136.0, 171.2, 172.9, 198.0; IR (KBr) 3334, 2918, 2850, 1732, 1684, 1649, 1543 cm^{-1}; LRMS (FAB) *m*/*z* 389 $[M]^+$. Anal. ($C_{23}H_{35}NO_4$) C, H, N. Chiral HPLC: Daicel Chiralpak AD, hexane/^{i}PrOH = 19/1, flow rate = 1.0 mL/min; t_R = 28.8 min (*R*), t_R = 23.6 min (*S*).

(2*R*)-2-Dodecanoylamino-4-oxo-4-phenylbutyric acid ethyl ester (5b): $[\alpha]\ ^{23}_D$ −58.5 (94% ee, *c* 1.02, $CHCl_3$); mp 50−51 °C; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, J = 6.8 Hz), 1.2−1.4 (m, 19H), 1.5−1.7 (m, 2H), 2.21 (dt, 2H, J = 2.4, 7.7 Hz), 3.60 (dd, 1H, J = 4.1, 18.3), 3.75 (dd, 1H, J = 4.1, 18.3), 4.21 (q, 2H, J = 7.1 Hz), 4.96 (dt, 1H, J = 4.1, 8.0 Hz), 6.61 (brd, 1H, J = 8.0 Hz), 7.47 (apparent t, 2H, J = 7.6 Hz), 7.60 (apparent t, 1H, J = 7.3 Hz), 7.94 (apparent d, 2H, J = 7.5 Hz); ^{13}C NMR ($CDCl_3$) δ 14.0, 14.1, 22.6, 25.5, 29.1, 29.3, 29.4, 29.5, 31.9, 36.5, 40.5, 48.2, 61.7, 128.1, 128.7, 133.7, 136.0, 171.2, 172.9, 198.0; IR (neat) 3310, 2925, 2855, 1742, 1682, 1657, 1529 cm^{-1}; LRMS (EI) *m*/*z* 403 $[M]^+$. Anal. ($C_{24}H_{37}NO_4$) C, H, N. HPLC: Daicel Chiralpak AD, hexane/^{i}PrOH = 19/1, flow rate = 1.0 mL/min; t_R = 21.9 min (*S*), t_R = 26.6 min (*R*).

(2*R*)-4-Oxo-4-phenyl-2-tridecanoylaminobutyric acid ethyl ester (5c): $[\alpha]\ ^{29}_D$ −53.1 (92% ee, *c* 0.960, $CHCl_3$); mp 51 °C; ^{1}H NMR ($CDCl_3$) δ 0.87 (t, 3H, J = 6.8 Hz), 1.15−1.35 (m, 21H), 1.55−1.70 (m, 2H), 2.19 (dt, 2H, J = 3.2, 7.6 Hz), 3.58 (dd, 1H, J = 4.0, 18.3 Hz), 3.74 (dd, 1H, J = 4.2, 18.3 Hz), 4.20 (q, 2H, J = 7.1 Hz), 4.95 (dt, 1H, J = 8.0, 3.9 Hz), 6.60 (brd, 1H, J = 8.0 Hz), 7.46 (apparent t, 2H, J = 7.8 Hz), 7.58 (apparent t, 1H, J = 7.4 Hz), 7.93 (apparent d, 2H, J = 7.3 Hz); ^{13}C NMR ($CDCl_3$) δ 14.0, 14.1, 22.6, 25.5, 29.1, 29.3, 29.4, 29.5, 29.6, 31.9, 36.5, 40.5, 48.2, 61.7, 128.1, 128.7, 133.7, 136.0, 171.2, 172.9, 198.0; IR (KBr) 3326, 2917, 2849, 1731, 1684, 1645, 1544 cm^{-1}; LRMS (EI) *m*/*z* 417 $[M]^+$. Anal. ($C_{25}H_{39}NO_4$) C, H, N. Chiral HPLC: Daicel Chiralpak AD, hexane/^{i}PrOH = 19/1, flow rate = 1.0 mL/min; t_R = 24.3 min (*R*), t_R = 20.0 min (*S*).

(2*R*)-4-Oxo-4-phenyl-2-tetradecanoylaminobutyric acid ethyl ester (5d): $[\alpha]\ ^{28}_D$ −52.3 (92% ee, *c* 0.995, $CHCl_3$); mp 54−55 °C; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, J = 6.8 Hz), 1.15−1.35 (m, 23H), 1.55−1.70 (m, 2H), 2.20 (dt, 2H, J = 3.4, 7.6 Hz), 3.59 (dd, 1H, J = 3.9, 18.3 Hz), 3.75 (dd, 1H, J = 4.2, 18.3 Hz), 4.21 (q, 2H, J = 7.2 Hz), 4.96 (dt, 1H, J = 8.1, 3.9

Hz), 6.60 (brd, 1H, J = 8.1 Hz), 7.48 (apparent t, 2H, J = 7.7 Hz), 7.60 (apparent t, 1H, J = 7.4 Hz), 7.94 (apparent d, 2H, J = 7.3 Hz); ^{13}C NMR ($CDCl_3$) δ 14.0, 14.1, 22.6, 25.5, 29.1, 29.3, 29.3, 29.4, 29.6, 29.6, 31.9, 36.5, 40.5, 48.2, 61.7, 128.1, 128.7, 133.7, 136.0, 171.2, 172.9, 198.0; IR (KBr) 3338, 2920, 2851, 1737, 1683, 1648, 1542 cm^{-1}; LRMS (EI) m/z 432 [M + H]$^+$. Anal. ($C_{26}H_{41}NO_4$) C, H, N. Chiral HPLC: Daicel Chiralpak AD, hexane/^{i}PrOH = 19/1, flow rate = 1.0 mL/min; t_R = 20.1 min (R), t_R = 26.0 min (S).

Synthesis of HPA-11 to -14 from 5a−d. To a solution of **5** (0.2 mmol) in THF (3 mL, freshly distilled from ketyl) or DME (3 mL, freshly distilled from ketyl) was added 1 M K-Selectride in THF (0.32 mL, 0.32 mmol) at −78 °C (THF) or −45 °C (DME). The mixture was stirred at the same temperature for 15 min, and then 1 M $LiBEt_3H$ in THF (0.66 mL, 0.66 mmol) was added. The reaction mixture was allowed to warm to room temperature and further stirred for 1 h. The reaction was quenched by careful addition of water and 30% aqueous H_2O_2. The mixture was extracted with AcOEt, and the extract was washed with saturated aqueous $NaHCO_3$. The combined aqueous layer was extracted with AcOEt, and the combined organic layer was washed with brine and dried over anhydrous Na_2SO_4. The solvents were evaporated, and the residue was purified by chromatography on silica gel to give HPA analogues (87−99% yield). These HPA analogues were purified by recrystallization from CH_2Cl_2/hexane to afford enantiomerically and diastereomerically pure products.

(1*R*,3*R*)-*N*-(3-Hydroxy-1-hydroxymethyl-3-phenylpropyl)decanamide (HPA-10, 6e): $[\alpha]^{19}_D$ −34.1 (>99% ee, *c* 0.62, $CHCl_3$); mp 82−83 °C; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, J = 6.8 Hz), 1.2−1.4 (m, 12H), 1.55−1.7 (m, 2H), 1.93 (ddd, 1H, J = 6.8, 8.8, 14.6 Hz), 2.05 (ddd, 1H, J = 3.4, 5.6, 14.6 Hz), 2.16 (t, 2H, J = 7.7 Hz), 3.21 (brs, 1H), 3.60−3.70 (m, 2H), 3.79 (brs, 1H), 4.0−4.1 (m, 1H), 4.81 (dd, 1H, J = 3.4, 8.9 Hz), 6.38 (brd, 1H, J = 6.3 Hz), 7.25−7.4 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.6, 25.7, 29.3, 29.3, 29.5, 31.8, 36.8, 40.7, 50.6, 65.8, 72.0, 125.5, 127.7, 128.6, 144.2, 174.3; IR (KBr) 3292, 2920, 2850, 1643, 1551 cm^{-1}. HRMS (FAB): exact mass calcd for $C_{20}H_{34}NO_3$ [M + H]$^+$, 336.2538. Found: 335.2538. Anal. ($C_{20}H_{33}NO_3$) C, H, N.

(1*R*,3*R*)-*N*-(3-Hydroxy-1-hydroxymethyl-3-phenylpropyl)undecanamide (HPA-11, 6a): $[\alpha]^{19}_D$ −37.6 (>99% ee, *c* 0.61, $CHCl_3$); mp 85−86 °C; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, J = 6.8 Hz), 1.2−1.4 (m, 14H), 1.55−1.7 (m, 2H), 1.93 (ddd, 1H, J = 6.8, 8.8, 14.6 Hz), 2.04 (ddd, 1H, J = 3.4, 5.6, 14.6 Hz), 2.16 (t, 2H, J = 7.7 Hz), 3.00 (brs, 2H), 3.60−3.70 (m, 2H), 4.00−4.10 (m, 1H), 4.80 (dd, 1H, J = 3.4, 9.0 Hz), 6.44 (brd, 1H, J = 6.4 Hz), 7.25−7.4 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 25.7, 29.3, 29.3, 29.5, 29.6, 31.9, 36.8, 40.7, 50.6, 65.7, 72.0, 125.5, 127.7, 128.6, 144.2, 174.4; IR (KBr) 3292, 2921, 2851, 1641, 1552 cm^{-1}. HRMS (FAB): exact mass calcd for $C_{21}H_{35}NO_3Na$ [M + Na]$^+$, 372.2514. Found: 372.2531. Anal. ($C_{21}H_{35}NO_3$) C, H, N. HPLC: Daicel Chiralpak, hexane/^{i}PrOH = 19/1, flow rate = 1.0 mL/min; t_R = 13.4 min (1*R*,3*R*), t_R = 17.9 min (1*S*,3*S*).

(1*R*,3*R*)-*N*-(3-Hydroxy-1-hydroxymethyl-3-phenylpropyl)dodecanamide (HPA-12, 1 = 6b): $[\alpha]^{15}_D$ −36.3 (>99% ee, *c* 0.505, $CHCl_3$); mp 90−91 °C; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, J = 6.8 Hz), 1.20−1.40 (m, 16H), 1.55−1.7 (m, 2H), 1.93 (ddd, 1H, J = 6.8, 8.8, 14.8 Hz), 2.03 (ddd, 1H, J = 3.4, 5.4, 14.8 Hz), 2.16 (t, 2H, J = 7.7 Hz), 3.21 (brs, 1H), 3.65−3.7 (m, 1H), 3.87 (brs, 1H), 4.0−4.1 (m, 1H), 4.81 (dd, 1H, J = 3.4, 8.3 Hz), 6.41 (brd, 1H, J = 6.5 Hz), 7.25−7.4 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 25.7, 29.3, 29.3, 29.3, 29.5, 29.6, 29.6, 31.9, 36.8, 40.7, 50.4, 65.5, 71.8, 125.5, 127.7, 128.5, 144.2, 174.3; IR (neat) 3293, 2919, 2849, 1643, 1551 cm^{-1}. HRMS (EI): exact mass calcd for $C_{22}H_{37}NO_3$ [M]$^+$, 363.2273. Found: 363.2279. Anal. ($C_{22}H_{37}NO_3$) C, H, N. HPLC: Daicel Chiralpak AD, hexane/^{i}PrOH = 19/1, flow rate = 1.0 mL/min; t_R = 11.4 min (1*R*,3*R*), t_R = 15.0 min (1*S*,3*S*).

(1*R*,3*R*)-*N*-(3-Hydroxy-1-hydroxymethyl-3-phenylpropyl)tridecanamide (HPA-13, 6c): $[\alpha]^{19}_D$ −34.1 (>99% ee, *c* 0.54, $CHCl_3$); mp 91−92 °C; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, J = 6.8 Hz), 1.2−1.4 (m, 18H), 1.55−1.7 (m, 2H), 1.93 (ddd, 1H, J = 6.8, 8.8, 14.6 Hz), 2.04 (ddd, 1H, J = 3.4, 5.6, 14.6 Hz), 2.17 (t, 2H, J = 7.6 Hz), 2.93 (brs, 2H), 3.6−3.7 (m, 2H), 4.0−4.1 (m, 1H), 4.81 (dd, 1H, J = 3.4, 9.0 Hz), 6.48 (brd, 1H, J = 5.9 Hz), 7.25−7.4 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 25.7, 29.3, 29.3, 29.5, 29.6, 29.6, 31.9, 36.8, 40.7, 50.6, 65.8, 72.0, 125.5, 127.7, 128.6, 144.2, 174.3; IR (KBr) 3292, 2919, 2849, 1642, 1551 cm^{-1}. HRMS (FAB): exact mass calcd for $C_{23}H_{39}NO_3Na$ [M + Na]$^+$, 400.2828. Found: 400.2832. Anal. ($C_{23}H_{39}NO_3$) C, H, N. HPLC: Daicel Chiralpak, hexane/^{i}PrOH = 19/1, flow rate = 1.0 mL/min; t_R = 12.4 min (1*R*,3*R*), t_R = 16.5 min (1*S*,3*S*).

(1*R*,3*R*)-*N*-(3-Hydroxy-1-hydroxymethyl-3-phenylpropyl)tetradecanamide (HPA-14, 6d): $[\alpha]^{19}_D$ −36.2 (>99% ee, *c* 0.36, $CHCl_3$); mp 94−95 °C; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, J = 6.8 Hz), 1.2−1.4 (m, 20H), 1.55−1.7 (m, 2H), 1.9−2.0 (m, 1H), 2.05 (ddd, 1H, J = 3.1, 5.0, 14.6 Hz), 2.16 (t, 2H, J = 7.6 Hz), 2.72 (brs, 2H), 3.6−3.7 (m, 2H), 4.0−4.1 (m, 1H), 4.81 (dd, 1H, J = 3.2, 8.8 Hz), 6.41 (brd, 1H, J = 5.6 Hz), 7.25−7.4 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 25.7, 29.3, 29.3, 29.5, 29.6, 29.6, 31.9, 36.8, 40.7, 50.6, 65.7, 72.0, 125.5, 127.7, 128.6, 144.2, 174.3; IR (KBr) 3294, 2919, 2849, 1643, 1550 cm^{-1}. HRMS (FAB): exact mass calcd for $C_{24}H_{42}NO_3$ [M + H]$^+$, 392.3165. Found: 392.3174. Anal. ($C_{24}H_{41}NO_3$) C, H, N. HPLC: Daicel Chiralpak AD + guard column, hexane/^{i}PrOH = 19/1, flow rate = 1.0 mL/min; t_R = 14.5 min (1*R*,3*R*), t_R = 18.9 min (1*S*,2*S*).

(1*R*,3*R*)-*N*-(3-Hydroxy-1-hydroxymethyl-3-phenylpropyl)pentadecanamide (HPA-15, 6f): $[\alpha]^{22}_D$ −31.5 (>99% ee, *c* 0.350, $CHCl_3$); mp 96−97.5 °C; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, J = 6.2 Hz), 1.2−1.4 (m, 22H), 1.55−1.65 (m, 2H), 1.85−2.1 (m, 2H), 2.17 (t, 2H, J = 7.3 Hz), 2.94 (brs, 2H), 3.6−3.75 (m, 2H), 4.0−4.1 (m, 1H), 4.81 (dd, 1H, J = 3.5, 8.8 Hz), 6.48 (d, 1H, J = 6.2 Hz), 7.2−7.4 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 25.7, 29.3, 29.3, 29.5, 29.6, 31.9, 36.8, 40.7, 50.6, 65.7, 72.0, 125.5, 127.7, 128.6, 144.2, 174.4; IR (neat) 3292, 2920, 2850, 1643, 1550 cm^{-1}. HRMS (FAB): exact mass calcd for $C_{25}H_{44}NO_3$ [M + H]$^+$, 406.3321. Found: 406.3322. Anal. ($C_{25}H_{43}NO_3$) C, H, N.

(1*R*,3*R*)-*N*-(3-Hydroxy-1-hydroxymethyl-3-phenylpropyl)hexadecanamide (HPA-16, 6g): $[\alpha]^{19}_D$ −33.0 (>99% ee, *c* 0.57, $CHCl_3$); mp 99−100 °C; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, J = 6.6 Hz), 1.2−1.4 (m, 24H), 1.55−1.7 (m, 2H), 1.94 (ddd, 1H, J = 6.9, 8.8, 14.9 Hz), 2.06 (ddd, 1H, J = 3.4, 5.1, 14.9 Hz), 2.17 (t, 2H, J = 7.6 Hz), 2.96 (brs, 1H), 3.55−3.8 (brs, 1H), 3.6−3.75 (m, 2H), 4.0−4.1 (m, 1H), 4.83 (dd, 1H, J = 3.4, 8.8 Hz), 6.36 (brd, 1H, J = 6.3 Hz), 7.25−7.4 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 25.7, 29.3, 29.4, 29.5, 29.7, 29.7, 29.7, 31.9, 36.9, 40.6, 50.7, 66.0, 72.2, 125.6, 127.8, 128.7, 144.2, 174.3; IR (KBr) 3294, 2919, 2849, 1643, 1550 cm^{-1}. HRMS (FAB): exact mass calcd for $C_{26}H_{46}NO_3$ [M + H]$^+$, 420.3477. Found: 420.3473. Anal. ($C_{26}H_{45}NO_3$) C, H, N.

(1*R*,3*R*)-*N*-(3-Hydroxy-1-hydroxymethyl-3-phenylpropyl)heptadecanamide (HPA-17, 6h): $[\alpha]^{19}_D$ −33.2 (>99% ee, *c* 0.205, $CHCl_3$); mp 101−102 °C; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, J = 6.7 Hz), 1.2−1.4 (m, 26H), 1.55−1.7 (m, 2H), 1.95 (ddd, 1H, J = 7.6, 8.9, 14.6 Hz), 2.07 (ddd, 1H, J = 3.4, 5.1, 14.6 Hz), 2.18 (t, 2H, J = 7.6 Hz), 2.81 (brs, 1H), 3.5−3.8 (brs, 1H), 3.65−3.75 (m, 2H), 4.0−4.1 (m, 1H), 4.84 (dd, 1H, J = 3.4, 8.9 Hz), 6.35 (brd, 1H, J = 6.1 Hz), 7.25−7.4 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 25.7, 29.3, 29.3, 29.4, 29.5, 29.6, 29.6, 29.7, 31.9, 36.8, 40.6, 50.7, 65.9, 72.1, 125.5, 127.8, 128.6, 144.2, 174.3; IR (KBr) 3294, 2919, 2849, 1643, 1550 cm^{-1}. HRMS (FAB): exact mass calcd for $C_{27}H_{48}NO_3$ [M + H]$^+$, 434.3634. Found: 434.3631.

(1*R*,3*R*)-*N*-(3-Hydroxy-1-hydroxymethyl-3-phenylpropyl)octadecanamide (HPA-18, 6i): $[\alpha]^{19}_D$ −34.0 (>99% ee, *c* 0.21, $CHCl_3$); mp 101−103 °C; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, J = 6.8 Hz), 1.2−1.4 (m, 28H), 1.55−1.7 (m, 2H), 1.95 (ddd, 1H, J = 7.1, 9.0, 14.8 Hz), 2.07 (ddd, 1H, J = 3.4, 5.0, 14.8 Hz), 2.17 (t, 2H, J = 7.6 Hz), 2.82 (brs, 1H), 3.58 (brs, 1H), 3.65−3.75 (m, 2H), 4.0−4.1 (m, 1H), 4.83 (dd, 1H, J = 3.4, 9.0 Hz), 6.33 (brd, 1H, J = 5.8 Hz), 7.25−7.4 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 25.7, 29.3, 29.4, 29.5, 29.6, 29.7, 29.7, 31.9, 36.9, 40.6, 50.8, 66.0, 72.3, 125.5, 127.9, 128.7, 144.2, 174.3; IR (KBr) 3294, 2919, 2849, 1643, 1550 cm^{-1}. HRMS

(FAB): exact mass calcd for $C_{28}H_{50}NO_3$ $[M + H]^+$, 448.3790. Found: 448.3789. Anal. ($C_{28}H_{50}NO_3$) C, H, N.

Methylation of HPA-12. To a mixture of HPA-12 (36.4 mg, 0.1 mmol) in iodomethane (1 mL) was added Ag_2O (70 mg, 0.3 mmol). The reaction mixture was stirred at room temperature for 12 h. The resultant mixture was filtered through a Celite pad, and the filtrate was concentrated in vacuo. The crude product obained was purified by chromatography on silica gel to give the desired **8** (20.1 mg) and **9** (9.0 mg) in 53% and 23% yield, respectively. The starting material, HPA-12, was also recovered in 13% yield.

(1*R*,3*R*)-(3-Hydroxy-1-methoxymethyl-3-phenylpropyl)dodecanamide (8): $[\alpha]^{19}_D$ −38.2 (>99% ee, *c* 0.78, $CHCl_3$); mp 40−41 °C; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, J = 6.8 Hz), 1.2−1.4 (m, 16H), 1.5−1.65 (m, 2H), 1.95−2.05 (m, 2H), 2.10 (dt, 2H, J = 2.0, 7.3 Hz), 3.36 (s, 3H), 3.42 (dd, 1H, J = 4.1, 9.5 Hz), 3.48 (dd, 1H, J = 3.7, 9.5 Hz), 3.62 (brs, 1H), 4.15−4.25 (m, 1H), 4.77 (dd, 1H, J = 4.8, 7.7 Hz), 5.95 (brd, 1H, J = 7.8 Hz), 7.2−7.4 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.6, 25.6, 29.2, 29.3, 29.3, 29.5, 29.6, 31.9, 36.8, 41.8, 47.1, 59.1, 71.8, 74.4, 125.6, 127.3, 128.3, 144.6, 173.3; IR (KBr) 3315, 2918, 2848, 1641, 1542 cm^{-1}. HRMS (FAB): exact mass calcd for $C_{23}H_{40}NO_3$ $[M + H]^+$, 378.3008. Found: 378.3010. Anal. ($C_{23}H_{39}NO_3$) C, H, N.

(1*R*,3*R*)-(3-Methoxy-1-methoxymethyl-3-phenylpropyl)dodecanamide (9): $[\alpha]^{19}_D$ −37.3 (>99% ee, *c* 0.41, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, J = 6.8 Hz), 1.2−1.4 (m, 16H), 1.55−1.7 (m, 2H), 1.88 (ddd, 1H, J = 5.2, 5.2, 14.4 Hz), 2.04 (ddd, 1H, J = 8.3, 8.3, 14.4 Hz), 2.16 (t, 2H, J = 7.7 Hz), 3.16 (s, 3H), 3.30 (s, 3H), 3.33 (dd, 1H, J = 4.4, 9.5 Hz), 3.38 (dd, 1H, J = 3.7, 9.5 Hz), 4.1−4.2 (m, 1H), 4.18 (dd, 1H, J = 5.1, 7.6 Hz), 5.87 (brd, 1H, J = 8.1 Hz), 7.25−7.4 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.6, 25.7, 29.3, 29.3, 29.5, 29.6, 29.6, 31.9, 37.0, 40.0, 47.3, 56.4, 59.0, 74.2, 82.0, 126.7, 127.7, 128.5, 141.8, 172.7; IR (neat) 3286, 2924, 2854, 1643, 1543 cm^{-1}. HRMS (FAB): exact mass calcd for $C_{24}H_{42}NO_3$ $[M + H]^+$, 392.3164. Found: 392.3173. Anal. ($C_{24}H_{41}NO_3$) C, H, N.

(1*R*,3*R*)-(3-Methoxy-1-hydroxymethyl-3-phenylpropyl)dodecanamide (10). To a solution of HPA-12 (75 mg, 0.206 mmol) and triethylamine (42 mg, 0.416 mmol) in dichloromethane (2.1 mL) was addedd benzoyl chloride (29 mg, 0.206 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 12 h, and then the reaction was quenched by $NaHCO_3$. The resultant mixture was extracted with dichloromethane three times, and the combined organic layer was washed with brine and dried over Na_2SO_4. The crude solution was concentrated in vacuo and purified by chromatography on silica gel to afford benzoic acid (2*R*,4*R*)-2-dodecanoylamino-4-hydroxy-4-phenylbutyl ester (87.3 mg) in 91% yield: mp 71−72 °C; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, J = 6.8 Hz), 1.2−1.4 (m, 16H), 1.5−1.65 (m, 2H), 2.05 (apparent t, 2H, J = 6.0 Hz), 2.11 (t, 2H, J = 7.4 Hz), 3.10 (brs, 1H), 4.3−4.35 (m, 1H), 4.4−4.55 (m, 2H), 4.86 (apparent t, 1H, J = 6.3 Hz), 6.00 (brd, 1H, J = 5.1 Hz), 7.25−7.4 (m, 5H), 7.44 (apparent t, 2H, J = 7.7 Hz), 7.58 (apparent t, 1H, J = 7.4 Hz), 8.01 (apparent d, 2H, J = 7.3 Hz); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 25.6, 29.2, 29.3, 29.4, 29.6, 29.6, 31.9, 36.9, 41.1, 47.3, 66.4, 72.1, 125.7, 127.7, 128.5, 128.6, 129.6, 129.7, 133.3, 144.2, 166.8, 173.6.

To a mixture of benzoic acid 2-dodecanoylamino-4-hydroxy-4-phenylbutyl ester (70.0 mg, 0.15 mmol) in iodomethane (5 mL) was added Ag_2O (348 mg, 1.5 mmol) at room temperature. The reaction mixture was stirred at room temperature for 2 days. The resultant mixture was filtered through a Celite pad, and the filtrate was concentrated in vacuo. The crude product obtained was purified by chromatography on silica gel to give the desired benzoic acid (2*R*,4*R*)-2-dodecanoylamino-4-methoxy-4-phenylbutyl ester (49.2 mg) in 68% yield: ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, J = 6.8 Hz), 1.2−1.4 (m, 16H), 1.55−1.7 (m, 2H), 1.94 (ddd, 1H, J = 4.9, 4.9, 14.4 Hz), 2.08 (ddd, 1H, J = 7.9, 7.9, 14.4 Hz), 2.17 (dt, 2H, J = 2.7, 7.7 Hz), 3.18 (s, 3H), 4.24 (dd, 2H, J = 4.9, 7.9 Hz), 4.30 (dd, 2H, J = 4.0, 10.9 Hz), 4.35−4.5 (m, 2H), 5.92 (brd, 1H, J = 7.6 Hz), 7.25−7.4 (m, 5H), 7.44 (apparent t, 2H, J = 7.7 Hz), 7.58 (apparent t, 1H, J = 7.4 Hz), 8.01 (apparent d, 2H, J = 7.3 Hz); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.6, 25.7, 29.3, 29.3, 29.4, 29.5, 29.6, 31.9, 37.0, 39.9, 47.0, 56.4, 66.3, 81.6, 126.6, 127.9, 128.4, 128.6, 129.6, 129.6, 133.1, 141.3, 166.5, 172.9.

To a solution of benzoic acid 2-dodecanoylamino-4-methoxy-4-phenylbutyl ester (24.6 mg, 0.051 mmol) in ethanol was added sodium ethoxide (3.5 mg, 0.051 mmol) at room temperature. The reaction mixture was stirred at room temperature for 10 h, and then the reaction was quenched with saturated ammonium chloride solution. The resulting mixture was extracted with ethyl acetate three times, and the combined organic layer was washed with brine and dried over Na_2SO_4. The crude solution was concentrated in vacuo and purified by chromatography on silica gel to afford (1*R*,3*R*)- (3-methoxy-1-hydroxymethyl-3-phenyl-propyl)dodecanamide (**10**) (18.6 mg) in 96% yield: $[\alpha]^{19}_D$ −38.6 (>99% ee, *c* 0.68, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, J = 6.8 Hz), 1.2−1.4 (m, 16H), 1.6−1.7 (m, 2H), 1.9−2.0 (m, 2H), 2.22 (t, 2H, J = 7.7 Hz), 3.20 (s, 3H), 3.55−3.65 (m, 2H), 3.95−4.1 (m, 2H), 4.24 (dd, 1H, J = 5.4, 7.1 Hz), 6.49 (brd, 1H, J = 5.6 Hz), 7.25−7.4 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 25.8, 29.3, 29.4, 29.5, 29.6, 31.9, 36.9, 39.6, 51.8, 56.4, 66.7, 82.1, 126.4, 128.0, 128.7, 141.2, 174.4; IR (neat) 3292, 2925, 2854, 1647, 1542 cm^{-1}. HRMS (FAB): exact mass calcd for $C_{23}H_{40}NO_3$ $[M + H]^+$, 378.3008. Found: 378.3023. Anal. ($C_{23}H_{39}NO_3$) C, H, N.

(2*R*,3*R*)-3-Benzyloxy-2-dodecanoylamino-4-oxo-4-phenylbutyric Acid Ethyl Ester (12). Compound **12** was prepared from **2b** and **11** by the Mannich-type reaction using the Cu(II)−chiral diamine **4** as mentioned above: $[\alpha]^{22}_D$ +12.2 (90% ee, *c* 1.58, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, J = 6.6 Hz), 1.15−1.35 (m, 19H), 1.50−1.60 (m, 2H), 2.15 (t, 2H, J = 7.3 Hz), 4.0−4.3 (m, 2H), 4.37 (d, 1H, J = 12.0 Hz), 4.81 (d, 1H, J = 12.0 H), 5.10 (dd, 1H, J = 2.0, 9.0 Hz), 5.43 (d, 1H, J = 2.0 Hz), 6.23 (d, 1H, J = 8.8 Hz), 7.2−7.4 (m, 5H), 7.47 (t, 2H, J = 7.8 Hz), 7.59 (t, 1H, J = 7.3 Hz), 7.90 (d, 2H, J = 7.3 Hz); ^{13}C NMR ($CDCl_3$) δ 14.0, 14.1, 22.6, 25.4, 29.0, 29.2, 29.3, 29.4, 29.5, 31.8, 36.2, 53.9, 62.0, 72.2, 78.7, 128.1, 128.2, 128.3, 128.4, 128.9, 133.8, 134.7, 136.6, 169.1, 173.1, 195.5; IR (neat) 3365, 2925, 2854, 1751, 1682, 1507 cm^{-1}. HRMS (EI): exact mass calcd for $C_{24}H_{36}NO_5$ $[M - Bn]^+$, 418.2593. Found: 418.2592. HPLC: Daicel Chiralpak AD + AD, hexane/^{i}PrOH = 19/1, flow rate = 0.95 mL/min; t_R = 34.0 min (2*R*,3*R*), t_R = 36.2 min (2*S*,3*S*).

(1*S*,2*R*,3*R*)-(2,3-Dihydroxy-1-hydroxymethyl-3-phenylpropyl)dodecanamide ((1*S*,2*R*,3*R*)-14). To a solution of **12** (95.3 mg, 0.187 mmol) in THF (2.8 mL) was added 1 M $LiBEt_3H$ (0.93 mL, 0.93 mmol) at 0 °C. The mixture was allowed to warm to room temperature and stirred for 1.5 h. The reaction was quenched by careful addition of water and 30% aqueous H_2O_2. The mixture was extracted with AcOEt, and the extract was washed with saturated aqueous $NaHCO_3$. The combined aqueous layer was extracted with AcOEt, and the combined organic layer was washed with brine and dried over anhydrous Na_2SO_4. The solvents were evaporated, and the residue was purified by chromatography on silica gel to give **13**. To a solution of **13** (43.2 mg, 0.092 mmol) in EtOH (1.8 mL) was added 20% $Pd(OH)_2$/C (43.2 mg). After replacement of argon by hydrogen, the mixture was stirred for 20 min at room temperature. $Pd(OH)_2$/C was filtered off, and the filtrate was concentrated in vacuo. The crude product was purified by chromatography on silica gel to afford (1*S*,2*R*,3*R*)-**14** (35% yield), (1*S*,2*R*,3*S*)-**14** (3% yield), and **15** (35% yield). The title compound, (1*S*,2*R*,3*R*)-**14**, could be also prepared from L-serine-derived diol ((1*S*,2*R*,3*R*)-**16**)[16] by the same method as the procedure described for (1*S*,2*S*,3*S*)-**14** (see below): $[\alpha]^{26}_D$ −5.2 (>99% ee, *c* 0.615, $CHCl_3$); mp 54−55 °C; ^{1}H NMR (CD_3OD) δ 0.90 (t, 3H, J = 6.4 Hz), 1.2−1.4 (m, 16H), 1.55−1.65 (m, 2H), 2.20−2.30 (m, 2H), 3.20−3.40 (m, 1H), 3.40−3.60 (m, 2H), 3.92 (dd, 1H, J = 1.8, 7.7 Hz), 4.51 (d, 1H, J = 7.9 Hz), 7.2−7.5 (m, 5H); ^{13}C NMR (CD_3OD) δ 15.3, 24.6, 27.9, 31.2, 31.3, 31.4, 31.5, 31.6, 33.9, 38.0, 53.6, 63.7, 75.4, 76.8, 129.2, 129.6, 130.1, 143.6, 177.0; IR (neat) 3735, 2925, 2854, 1646, 1541, 1456, 1199, 1086, 1086, 1051, 843, 763, 701 cm^{-1}; LRMS (EI) *m*/*z* 380 $[M + H]^+$. Anal. ($C_{22}H_{37}NO_4$) C, H, N.

(1*S*,2*S*,3*S*)-(2,3-Dihydroxy-1-hydroxymethyl-3-phenylpropyl)dodecanamide ((1*S*,2*S*,3*S*)-14). Compound **16** was prepared according to the literature procedure.[16] To a solution of **16** (109.0 mg, 0.32 mmol) in MeOH (1.5 mL) was added 4 N HCl−AcOEt (1.5 mL). The reaction mixture was refluxed for 1 h and cooled to room temperature. The mixture was concentrated in vacuo to afford crude product 3-amino-1-phenylbutane-1,2,4-triol hydrochloride. To this flask was added THF (5.0 mL), Et_3N (65.4 mg, 0.64 mmol), and dodecanoic acid *p*-nitrophenol ester (207.6 mg, 0.64 mmol), successively. The reaction mixture was stirred at room temperature for 8 h, and the reaction was quenched by adding saturated aqueous $NaHCO_3$ and 1 N aqueous NaOH. The mixture was extracted with CH_2Cl_2 five times, and the organic layer was washed with brine and dried over anhydrous Na_2SO_4. The solvent was evaporated and the residue was purified by chromatography on silica gel to afford (1*S*,2*S*,3*S*)-**14** (77.4 mg, 63% yield in two steps): $[\alpha]^{21}_D$ +16.3 (>99% ee, *c* 1.005, $CHCl_3$); mp 122−123 °C; ^{1}H NMR (CD_3 OD) δ 0.93 (t, 3H, J = 6.6 Hz), 1.30−1.50 (m, 16H), 1.60−1.70 (m, 2H), 2.27 (t, 2H, J = 7.5 Hz), 3.75 (dd, 1H, J = 2.9, 7.9 Hz), 3.81 (d, 2H, J = 4.9 Hz), 4.03 (dd, 1H, J = 4.9, 7.9 Hz), 4.73 (d, 1H, J = 2.9 Hz), 7.20−7.50 (m, 5H); ^{13}C NMR (CD_3 OD) δ 15.3, 24.6, 27.9, 31.2, 31.3, 31.4, 31.5, 31.6, 33.9, 38.0, 55.4, 63.1, 74.9, 76.5, 129.4, 128.6, 129.0, 129.9, 144.4, 177.7; IR (neat) 3303, 2920, 2850, 1631, 1541 cm^{-1}. HRMS (FAB): exact mass calcd for $C_{22}H_{38}NO_4$ $[M + H]^+$, 380.2801. Found: 380.2795. Anal. ($C_{22}H_{37}NO_4$) C, H, N

(2,3-Dihydroxy-1-hydroxymethyl-3-phenylpropyl)carbamic Acid *tert*-Butyl Ester (18 and 19). Compound **17** was prepared according to the literature procedure.[16] To a solution of **17** (70.9 mg, 0.27 mmol) and 50% aqueous solution of NMO (81.0 mg, 0.35 mmol) in H_2O/acetone/CH_3CN (1:1:1, 2.7 mL) was added microencapsulated OsO_4 (5 mol %).[17] The reaction mixture was stirred at room temperature for 36 h. After the reaction was completed, methanol was added to the mixture, and the catalyst was separated by filtration and washed with methanol. The filtrate was concentrated in vacuo, and saturated aqueous Na_2SO_3 was added to the residue. The mixture was extracted with CH_2Cl_2 four times, and the combined organic layer was washed with brine and dried over anhydrous Na_2SO_4. The solvent was evaporated, and the residue was purified by chromatography on silica gel to afford **18** (50.6 mg, 63% yield) and **19** (23.3 mg, 29% yield). ^{1}H and ^{13}C NMR spectra of **18** and **19** were identical with the reported ones.[16]

(1*S*,2*R*,3*S*)-(2,3-Dihydroxy-1-hydroxymethyl-3-phenylpropyl)dodecanamide ((1*S*,2*R*,3*S*)-14). Preparation of (1*S*,2*R*,3*S*)-**14** from **18** was accomplished by the same method as described for the synthesis of (1*S*,2*S*,3*S*)-**14** from **16**. The yield was 74% in two steps: $[\alpha]^{26}_D$ −32.5 (>99% ee, *c* 1.145, $CHCl_3$); mp 53−54 °C; ^{1}H NMR (CD_3 OD) δ 0.93 (t, 3H, J = 6.2 Hz), 1.30−1.45 (m, 16H), 1.60−1.75 (m, 2H), 2.30 (t, 2H, J = 7.1 Hz), 3.60−3.75 (m, 2H), 3.87 (dd, 1H, J = 1.3, 8.4 Hz), 4.30−4.40 (m, 2H), 7.25−7.45 (m, 5H); ^{13}C NMR (CD_3 OD) δ 15.3, 24.6, 27.9, 31.2, 31.4, 31.5, 31.6, 33.9, 37.9, 54.2, 63.8, 75.3, 76.4, 129.4, 129.4, 129.9, 144.4, 178.2; IR (neat) 3335, 2925, 2851, 1635, 1535 cm^{-1}. HRMS (EI): exact mass calcd for $C_{22}H_{37}NO_4$ $[M]^+$, 379.2723. Found: 379.2729. Anal. ($C_{22}H_{37}NO_4$) C, H, N.

(1*S*,2*S*,3*R*)-(2,3-Dihydroxy-1-hydroxymethyl-3-phenylpropyl)dodecanamide ((1*S*,2*S*,3*R*)-14). Preparation of (1*S*,2*S*,3*R*)-**14** from **19** was accomplished by the same method as described for the synthesis of (1*S*,2*S*,3*S*)-**14** from **16**. The yield was 70% in two steps: $[\alpha]^{22}_D$ −27.4 (>99% ee, *c* 0.250, $CHCl_3$); mp 82−83 °C; ^{1}H NMR (CD_3 OD) δ 0.93 (t, 3H, J = 6.5 Hz), 1.30−1.50 (m, 16H), 1.60−1.70 (m, 2H), 2.22 (t, 2H, J = 7.3 Hz), 3.75−3.85 (m, 2H), 3.93 (apparent t, 1H, J = 5.7 Hz), 4.10 (apparent q, 1H, J = 5.1 Hz), 4.68 (d, 1H, J = 6.2 Hz), 7.20−7.50 (m, 5H); ^{13}C NMR (CD_3OD) δ 15.3, 24.6, 27.8, 31.2, 31.3, 31.4, 31.5, 31.6, 33.9, 38.1, 54.5, 62.8, 76.8, 77.3, 129.3, 129.9, 144.1, 177.0; IR (neat) 3305, 2920, 2850, 1643, 1543 cm^{-1}. HRMS (FAB): exact mass calcd for $C_{22}H_{38}NO_4$ $[M + H]^+$, 380.2801. Found: 380.2787. Anal. ($C_{22}H_{37}NO_4$) C, H, N.

Biological Assays. The effect of HPA-12 and its derivatives on SM biosynthesis was examined by metabolic labeling with radioactive serine in CHO-K1 cells as described previously.[10] In brief, the subconfluent CHO cell monolayers in 60 mm dishes were preincubated in 1.5 mL of serum-free medium containing HPA-12 or its derivatives at various concentrations up to 1 μM at 4 °C for 15 min. The concentration of DMSO, the vehicle of the drug stock solutions, during the preincubation was adjusted to 0.01% (v/v) in each dish. After addition of L-[^{14}C]serine (0.75 μCi), the cells were incubated at 33 °C for 2 h. As the drug-minus control, cells were incubated with 0.01% (v/v) DMSO in the absence of the HPA derivatives. Then, lipids were extracted from the cells and separated by TLC. Radioactive lipids separated on the plates were analyzed with a BAS 2000 image analyzer (Fuji Film, Inc., Tokyo). The radioactivity of metabolically labeled SM was corrected to the protein amount of the cell lysate used for lipid extraction. The corrected radioactivity was used as a measure of de novo SM synthesis in cells. The level of SM synthesis in the drug-minus control was taken as 100%.

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