Synthesis and Immunobiological Activity of an Original Series of Acyclic Lipid A Mimics Based on a Pseudodipeptide Backbone

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N^{δ}-L-Homoserinyl-D-ornithinol pseudodipeptides *N*-acylated with typical *Escherichia coli* lipid A fatty acid residues and mono-*O*- or bis-*O*-phosphorylated have been prepared and their properties investigated. The derivatives carrying two phosphate groups were found to be inducers of NO production. In addition, while they were unable to induce significantly the production of interleukin-6 (IL-6) by human PBMC cells, these compounds behaved also as potent antagonists of LPS-induced IL-6 production in the same human cells system. In conclusion, the molecules described here are the first members of an original class of immunobiologically active lipid A mimics based on an acyclic pseudodipeptide backbone carrying only the essential functionalities of the parent lipid A structure (OM-174). As the products exhibit very low endotoxicity and pyrogenicity, this class of lipid A mimics therefore opens a new generation of immunoadjuvants that possibly could reach clinical applications.

Introduction

Lipopolysaccharides (LPS) are amphiphilic macromolecules expressed at the outer membrane of Gram-negative bacteria that possess extremely potent immunostimulating activities.[1] LPS ("endotoxins") are responsible for the pathogenesis and manifestation of Gram-negative infections and, ultimately, for septic shock.[2] Several years ago it was well established that most of the biological activities of these lipopolysaccharides arise from their terminal lipophilic fragment, known as lipid A,[3] which serves as the LPS membrane anchor. While large structural variations exist in the polysaccharide part of LPS, in particular in the antigenic O-specific chain, the molecular structure of lipid A is relatively conserved across Gram-negative bacteria: it is generally constituted of a β-(1→6)-linked glucosamine disaccharide backbone carrying two phosphate groups at O-1 and O-4′ and six or more fatty acyl groups linked as esters and amides (e.g. *Escherichia coli* lipid A; see Figure 1).[3]

Lipid A is endowed with an overwhelming spectrum of biological activities (activation of macrophages, B cell mitogenicity, enhancement of host resistance against bacterial or viral infections and against tumors, etc.)[3,4a] and, since its discovery, stimulated enormous interest from both industrial and academic research laboratories. In particular, much effort has been dedicated to modifications of the lipid A structures with the goal of reducing the natural endotoxicity of the parent compound while maintaining or improving its beneficial immunostimulating properties or of identifying nontoxic antagonists for the treatment of septicemia. Several lipid A *derivatives* are currently under investigation;[4,5] for example, monophosphorylated lipid A (MPL)[6] is considerably less toxic than the parent diphosphate and has been the object of extensive studies, in particular as a vaccine adjuvant.[7] To date, GlaxoSmithKline Biologicals (GSK Bio) has received European approval for its hepatitis B vaccine,

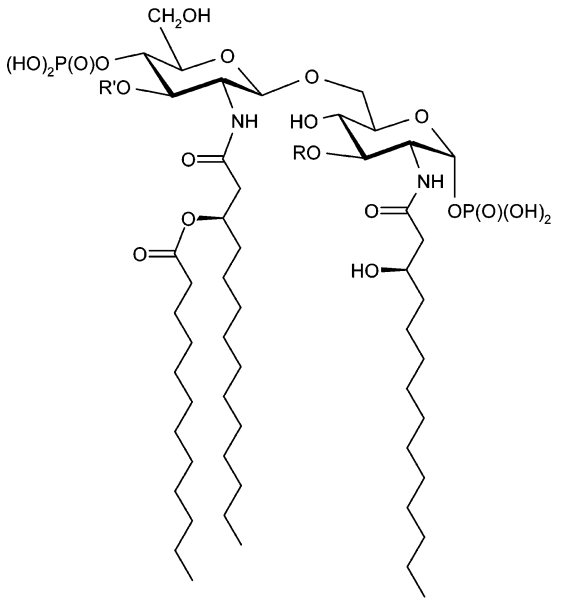

Figure 1. Structure of *E. coli* lipid A and of OM-174.

Fendrix, containing Corixa's MPL adjuvant. MPL adjuvant is now incorporated in two approved products and in multiple vaccines nearing approval around the world. Two other late-stage GSK Bio vaccines that contain MPL include Simplirix, a vaccine for genital herpes, and Cervarix, a cervical cancer vaccine that targets the human papillomavirus. Additionnal GSK Bio vaccines containing MPL include a malaria vaccine, which recently completed a phase IIb trial, as well as vaccines for tuberculosis, prostate cancer, and breast cancer. Also, modified lipid A's whose structure is derived from that of naturally occurring nontoxic lipid A's (from *Rhodobacter capsulatus* and *Rhodobacter sphaeorides*) have been developed as potent endotoxin antagonists.[5b−d]

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In the early 90s, a new lipid A derivative (OM-174, Figure 1) was isolated by OM PHARMA from partially degraded *E. coli* LPS.[8] This derivative has lost both sugar-*O*-acyl substituents and therefore carries only the *N*-linked fatty acid residues *of E. coli* lipid A, namely a (*R*)-3-hydroxytetradecanoyl group at *N*-2 and a (*R*)-3-dodecanoyloxytetradecanoyl group at *N*-2′, thus leaving only three long-chain acyl groups on the structure. Thorough pharmacological investigations of this new compound revealed that it has potent antitumor activity in several in vivo tumor models[9] and that it is an effective immunoadjuvant with very low toxicity. These remarkable biological activities provided evidence that the presence of six fatty acid residues is not a necessary condition for lipid A derivatives to exhibit useful immunostimulatory properties.[10]

The high interest of OM-174 and related compounds as potential therapeutic agents, combined with the fact that such structures are accessible only by way of lengthy syntheses,[11] prompted us to design and develop simplified structures carrying the main functionalities of the parent product. We report in this paper the synthesis of a novel series of lipid A mimics based on an acyclic pseudodipeptide backbone and the evaluation of their immunostimulating activity using *E. coli* LPS as a reference. These compounds constitute the first examples of a new class of highly active nontoxic lipid A-mimicking structures.

Structural Design and Synthetic Planning. A simplification of the lipid A structure that has been extensively studied is the replacement of the "reducing" glucosamine residue of the disaccharide scaffold by an acylated acyclic aglycon.[4b,10,12,13] In these analogues, the aglycon is generally a glycosidically linked hydroxy amino acid derivative *N*-acylated with a fatty acid. Interesting immunostimulating activities were found for several of the analogues investigated,[10b] which suggested that the functional groups of the reducing sugar moiety of lipid A are not necessary for biological activity. Further simplification of the structure led to deceptively simple analogues such as the *O*,*N*-bis-acylated derivative of 4-amino-5-hydroxypentanoic acid SDZ 280.961[14] [*O*-(*R*)-3-tetradecanoyloxytetradecanoyl *N*-(*R*)-3-hydroxytetradecanoyl (4*S*)-4-amino-5-hydroxypentanoic acid], a compound designed to mimic the reducing sugar component of lipid A which effectively stimulates TNF-α release from human peripheral blood mononuclear cells (PBMC). Bacterial ornithine-containing lipids, recently shown to have macrophage-activating properties,[15] can also be considered as belonging to this class of simple lipid A analogues. A fully acyclic analogue of *E. coli* lipid A, constituted by a symmetric phospholipid dimer connected to a non-carbohydrate backbone and carrying six fatty acid chains (ER 112022), was recently developed;[16] this compound was found to interact directly with the LPS receptor and thus to behave as a potent lipid A agonist.[17]

We have designed novel acyclic analogues of lipid A based on the OM-174 model and on the following considerations: (1) replacement of the two sugar units by amino acid-derived elements carrying anchoring functions at the appropriate location; (2) replacement of the glycosidic linkage by an amide bond; (3) conservation of the essential functions of the lipid A model directly exposed to a putative receptor, namely, the phosphate groups and the fatty acid chains; and (4) conservation of the amphiphilic character of the parent lipid A model.

These considerations led to the design of a general structure of type **I** (Figure 2). Considering appropriate disconnections, such structure should be readily accessible from the building blocks D/L-homoserine, D/L-ornithinol, or D/L-lysinol; substituted fatty acids; and phosphorylating agents. We set out as goals

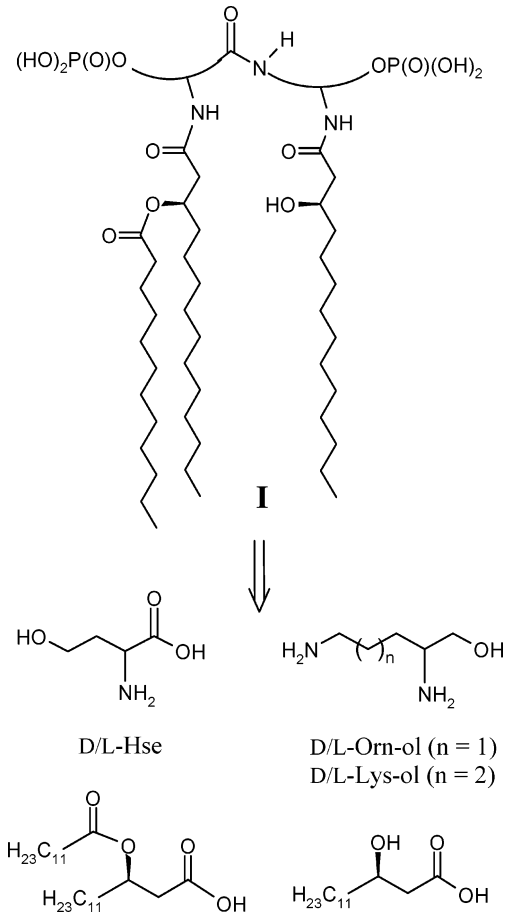
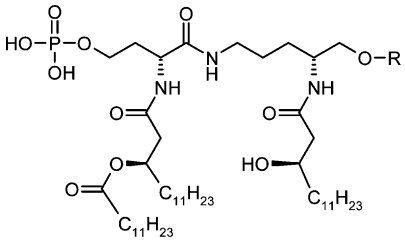


Figure 2. Proposed structure and synthetic planning.



1 (OM-294-DP) R = $P(O)(OH)_2$
2 (OM-294-MP) R = H

Figure 3. Target lipid A mimics.

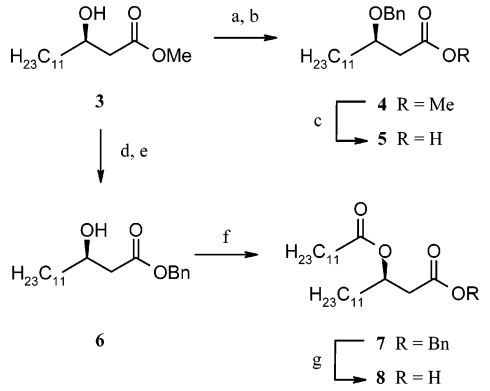
the preparation of the D-ornithinol-derived structure **1** (OM-294-DP)[18] as well as of the monophosphorylated derivative **2** (OM-294-MP),[18] an analogue that is of interest because of its relation to monophosphorylated lipid A (Figure 3). The resulting structures were synthesized as described below and were found to exhibit biological activities similar to those of the parent lipid A, with extremely low endotoxicity.

Results and Discussion

Synthetic Studies. To develop a convergent synthesis, it was decided to perform the amide bond coupling late in the synthesis using completely elaborated building blocks carrying both the fatty acyl group and a protected phosphate. These building blocks would arise in initial studies from DL-homoserine and from D-ornithine.

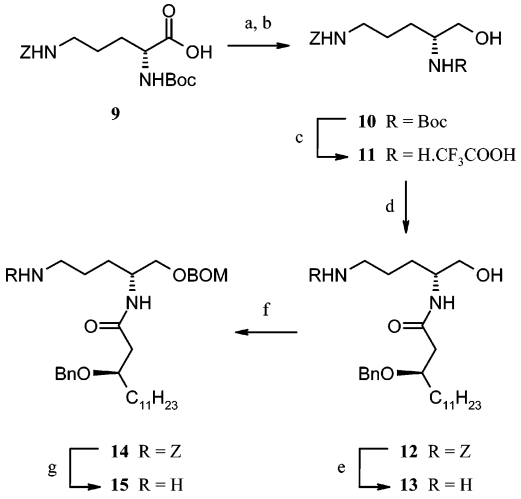
Synthesis of Fatty Acids (Scheme 1). The known (*R*)-3-benzyloxy- and (*R*)-3-dodecanoyloxytetradecanoic acid derivatives **5** and **8** were prepared by modified or improved proce-

Scheme 1[a]



[a] Reagents and conditions: (a) TMSCl, Et_3N, THF; (b) PhCHO, TMSOTf, Et_3SiH, CH_2Cl_2; (c) LiOH, THF−water, 50 °C; (d) 10 N NaOH, EtOH; (e) BnBr, Et_3N, Bu_4NI, EtOAc; (f) $C_{11}H_{23}COCl$, pyridine, CH_2Cl_2; (g) Pd/C, H_2, EtOAc−EtOH.

Scheme 2[a]



[a] Reagents and conditions: (a) *i*-BuOCOCl, *N*-methylmorpholine, THF, −15 °C; (b) $NaBH_4$, H_2O, −15 °C, 10 min; (c) TFA; (d) acid **5**, *i*-BuOCOCl, *N*-methylmorpholine, THF, −15 °C then amine **11**, Et_3N, THF; (e) Pd/C, H_2, EtOH−Et_3N; (f) BOMCl, $(i\text{-Pr})_2NEt$, CH_2Cl_2; (g) $Pd(OH)_2/C$, H_2, EtOH−Et_3N.

dures.[18] The benzylation of base labile 3-hydroxyalkanoates, including methyl 3-hydroxytetradecanoate **3**,[19] has been achieved using Ag_2O−BnBr[20a] or benzyl trichloroacetimidate;[20b,c] however, the yield of these reactions does not exceed 60−80%, and the product is difficult to purify. Compound **3** was therefore benzylated by way of its trimethylsilyl ether under the reductive conditions described by Nishizawa;[21] these conditions provided the desired product **4**[20c] in very high yield, which could be engaged in the subsequent saponification without purification to give pure **5** [24] in 81% yield from **3**.

Saponification of **3** afforded (*R*)-3-hydroxytetradecanoic acid, which was converted into its benzyl ester **6**[22] in essentially quantitative yield by reaction with benzyl bromide and triethylamine; the crude benzyl ester was esterified with dodecanoyl chloride to give compound **7**, which was purified and submitted to hydrogenolysis to afford pure (*R*)-3-dodecanoyloxytetradecanoic acid[23,24] **8** in 86% yield from (*R*)-3-hydroxytetradecanoic acid.

Synthesis from DL-Homoserine. The α-Boc-δ-Z-D-ornithine derivative (**9**) was converted into α-Boc-δ-Z-D-ornithinol[25] **10** using the method reported by Martinez[26] (Scheme 2). The N-Boc group was then cleaved to give **11**, and the α-amino group of D-ornithinol could be acylated with (*R*)-3-benzyloxytetradecanoic acid **5** to give **12** in 69% yield (mixed anhydride technique). The Z group was then selectively cleaved in the presence of the benzyl ether by catalytic hydrogenolysis in the presence of triethylamine[27] to give the amine building block **13**.

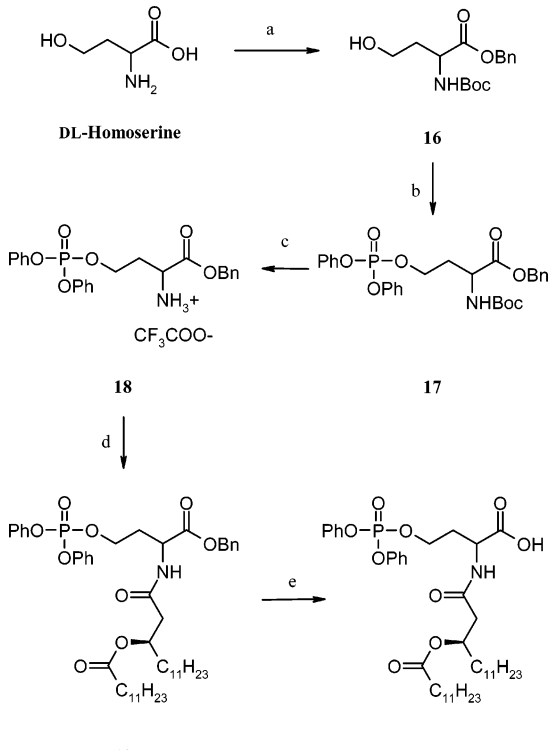
The *O*-diphenylphosphoryl derivative of **12** was also prepared; however, the amine obtained after cleavage of the Z group was not sufficiently stable to be engaged in amide-bond-forming reactions. Phosphorylation at that position was therefore postponed to a later stage of the synthesis.

For further studies, the free hydroxyl group of **12** was also protected as a benzyloxymethyl (BOM) ether, and the amino group of the resulting ornithinol derivative **14** was deprotected under the same conditions as **12** to give **15**. It is noteworthy that both the benzyl and the benzyloxymethyl ether functions were unaffected by the hydrogenolysis in the presence of a tertiary amine.

For the synthesis of the homoserine derivative **20** (Scheme 3), the free racemic amino acid was first submitted to a three-step sequence without isolation of the intermediates to form derivative **17**: conversion into the cesium carboxylate, formation of the *N*-Boc derivative immediately followed by formation of the benzyl ester by alkylation of the cesium carboxylate,[28] and phosphorylation of the remaining free OH group using $(PhO)_2P(O)Cl$.[29] By this sequence, compound **17** was obtained in 82% overall yield and the formation of the corresponding lactone was avoided.

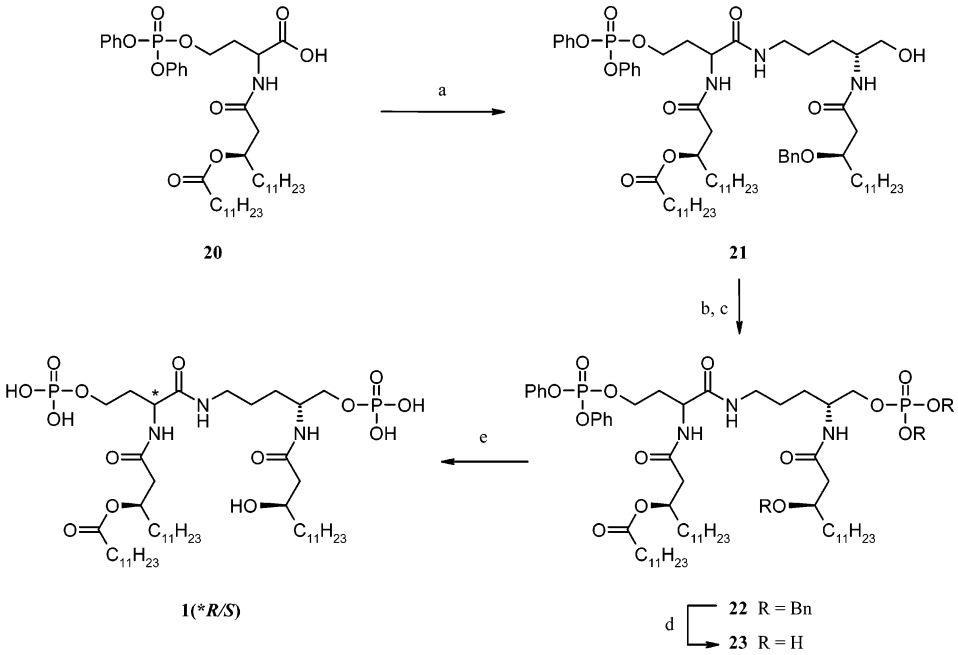
The N-Boc group was then cleaved under standard conditions and the amino group of **18** was acylated with (*R*)-3-dodecanoyloxytetradecanoic acid **8** to give the key intermediate **19**. The benzyl ester function of **19** was cleaved under mild hydrogenolytic conditions just prior to the next reaction because of the lability of the resulting free acid **20**.

Scheme 3[a]



[a] Reagents and conditions: (a) (i)Cs_2CO_3, N NaOH; (ii) $(Boc)_2O$, dioxane; (iii) BnBr, DMF; (b) $(PhO)_2P(O)Cl$, DMAP, pyridine, CH_2Cl_2; (c) TFA; (d) acid **8**, *i*-BuOCOCl, *N*-methylmorpholine, THF, −15 °C, 30 min then amine **18**, Et_3N, THF; (e) Pd/C, H_2, EtOH.

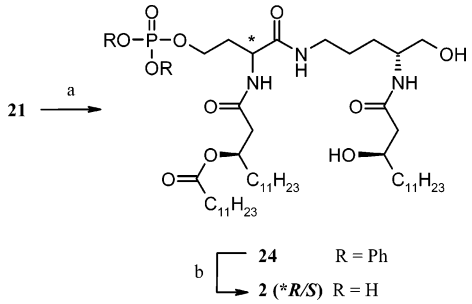
Scheme 4[a]



20 **21** **1(*R/S*)**

[a] Reagents and conditions: (a) IIDQ, CH_2Cl_2, 15 min then amine **13**, CH_2Cl_2; (b) $(BnO)_2PNEt_2$, 1*H*-tetrazole, THF; (c) *m*CPBA, CH_2Cl_2; (d) Pd/C, H_2, EtOH; (e) PtO_2, H_2, EtOH.

Scheme 5[a]



[a] Reagents and conditions: (a) Pd/C, H_2, EtOH−AcOH; (b) PtO_2, H_2, EtOH.

The amide bond formation between fragments **13** and **20** was investigated under a variety of conditions. The desired pseudodipeptide **21** could be obtained in 56% yield using IIDQ (2-isobutyloxy-1-isobutyloxycarbonyl-1,2-dihydroquinoline)[30] as the coupling agent (Scheme 4). Compound **21** was then phosphorylated by way of the phosphoramidite technique,[31] to give derivative **22**, which was then deprotected by two successive hydrogenolyses, the first one in the presence of Pd on charcoal to cleave the benzyl groups, and the second one in the presence of platinum oxide to cleave the phenyl phosphates.[32] This sequence thus provided the diphosphorylated pseudodipeptidolipid **1** as a mixture of stereoisomers at C-2b. Similarly, the monophosphorylated analogue **2** was prepared by the deprotection of the amide precursor **21** (Scheme 5).

Synthesis from D- or L-Aspartic Acid. Because of the difficulties associated with the homoserine-derived fragments, and the high cost of enantiomerically pure Hse, the synthetic strategy was modified. It was envisioned that the homoserine component of the final products could arise from an aspartic acid residue[33] and be generated by a reduction process after the amide-forming reaction. Thus β-benzyl D-aspartate was *N*-acylated with fatty acid **8** to afford compound **25** in high yield (mixed anhydride method) (Scheme 6) and this product was coupled to amine **13** in the presence of IIDQ. Careful analysis of the resulting coupling product **26** revealed however that a considerable amount of α-epimerization (about 30%) had taken place during the reaction. Epimerization could be reduced to about 13% by carrying the reaction at lower temperature (0 °C); better results, however, were obtained when the coupling was performed using diisopropylcarbodiimide in the presence of HOAt[34] (∼2.5% epimerization at C-2b, 72% yield). The reduction of the β-carboxyl function of **26** was then investigated. The direct reduction of **26** with $NaBH_4$ gave in modest yield a product resulting from the reductive opening of an intermediate cyclic imide (see below). The reduction was successfully achieved by way of the mixed anhydride method:[26] selective cleavage of the β-benzyl ester of **26** followed by formation of the mixed anhydride with isobutyl chloroformate and rapid treatment of this intermediate with $NaBH_4$ provided the expected diol **29** in 74% yield. This compound was then bis-phosphorylated and the resulting product **30** was deprotected by hydrogenolysis to afford the product **1-(*R*)** (=2b(*R*)).

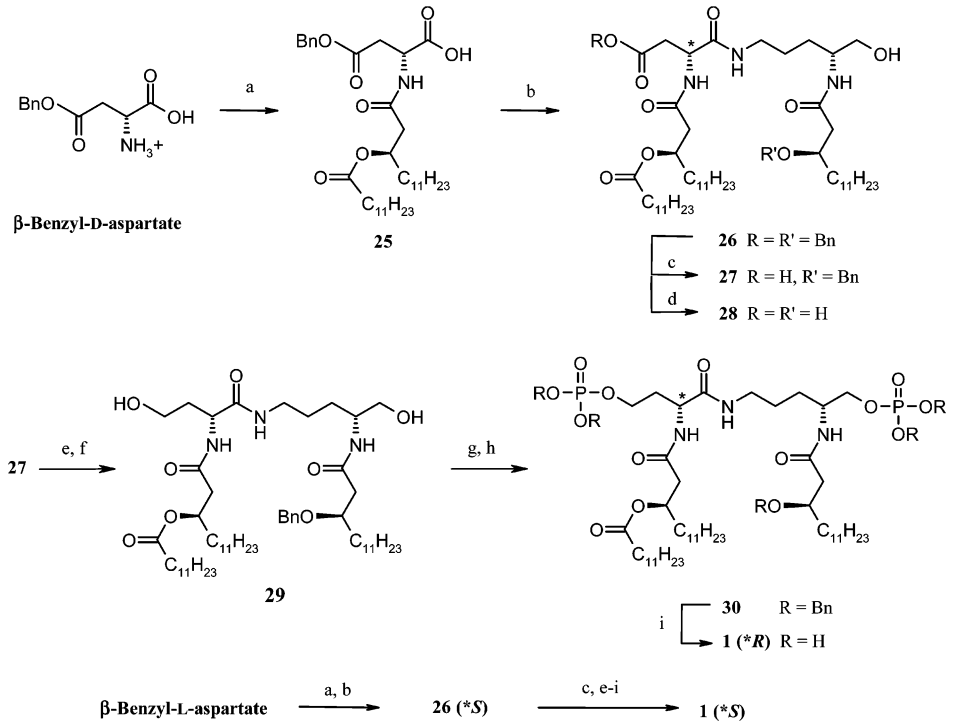
The same sequence of reaction was applied to the synthesis of the **1(*S*)**-epimer starting form β-benzyl-L-aspartate, by way of the (*S)-epimer of intermediate **26**.

The monophosphorylated analogue **2(*R*)** was also obtained by this strategy: compound **25** was coupled with the *O*-benzyloxymethyl ornithinol derivative **15**, to give compound **31** (Scheme 7), which was converted into **33** by the same reductive process and then *O*-phosphorylated and deprotected to give **2(*R*)**.

The same sequence of reaction was applied to the synthesis of the **2(*S*)**-epimer starting form β-benzyl-L-aspartate, by way of the (*S)-epimer of intermediate **31**.

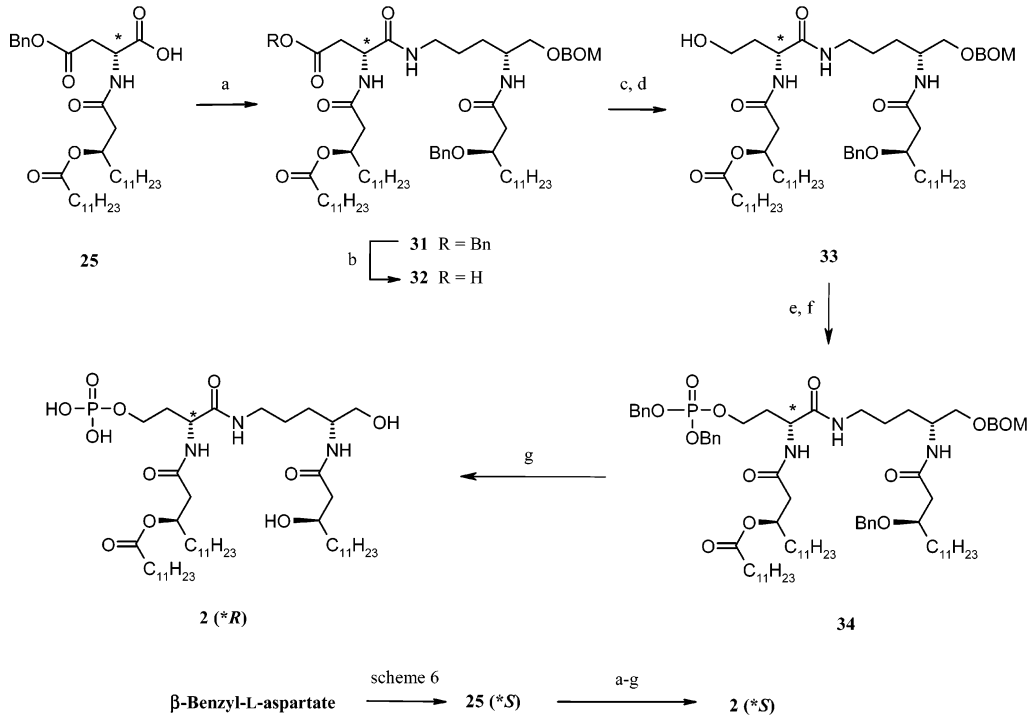
As indicated above, the treatment of **26** [∼3:1 mixture of 2b(*R*)- and 2b(*S*)-epimers] with $NaBH_4$ gave, in 43% yield, an unexpected product having a β-acyloxyacylamido group in the fragment arising from the aspartate (compound **35**, Scheme 8). This compound was formed undoubtedly by way of a cyclic imide, which underwent regioselective reductive opening to give

Scheme 6[a]



[a] Reagents and conditions: (a) acid **8**, *i*-BuOCOCl, *N*-methylmorpholine, THF, −15 °C, 30 min then H-D-Asp(OBn)-OH, CH_3CN-H_2O, Et_3N; (b) amine **13**, HOAt, *N*,*N*′-diisopropylcarbodiimide, CH_2Cl_2; (c) Pd/C, H_2, EtOH−EtOAc, Et_3N; (d) Pd/C, H_2, EtOH−EtOAc; (e) *i*-BuOCOCl, *N*-methylmorpholine, THF; (f) $NaBH_4$, H_2O, rt, 5 min; (g) $(BnO)_2PNEt_2$, 1*H*-tetrazole, THF; (h) *m*CPBA, CH_2Cl_2; (i) Pd/C, H_2, EtOH.

Scheme 7[a]

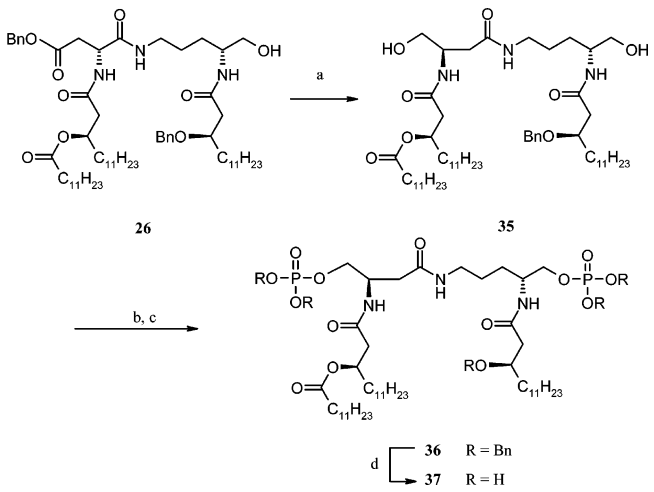


[a] Reagents and conditions: (a) IIDQ, CH_2Cl_2, 15 min then amine **15**, CH_2Cl_2; (b) Pd/C, H_2, EtOH−EtOAc, Et_3N; (c) *i*-BuOCOCl, *N*-methylmorpholine, THF, 30 min; (d) $NaBH_4$, H_2O, 5 min; (e) $(BnO)_2PNEt_2$, 1*H*-tetrazole, THF; (f) *m*CPBA, CH_2Cl_2; (g) Pd/C, H_2, EtOH−EtOAc.

the rearranged product **35**. As this structure is of interest for structure−activity studies, compound **35** was elaborated into the bis-phosphorylated pseudodipeptide **37** by a similar sequence of reactions.

Biological Studies. Endotoxicity. The endotoxicity of compounds **1(*R/S)**, **1(*R)**, **1(*S)** and **2(*R/S)**, **2(*R)**, and **2(*S)** was evaluated by the LAL (*Limulus* amoebocyte lysate) chromogenic assay.[35] This very sensitive assay is based on the phenomenon that endotoxins induce coagulation of the blood of the horseshoe crab (*Limulus polyphemus*). The results (Table 1) indicate that solutions of 0.1 mg/mL of the compounds of the series **1** and **2** exhibit a low endotoxicity pattern, compounds of the series **1** being even less endotoxic than compounds of series **2**.

Scheme 8[a]



[a] Reagents and conditions: (a) $NaBH_4$, MeOH−H_2O, −15 °C → rt; (b) $(BnO)_2PNEt_2$, 1*H*-tetrazole, THF; (c) *m*CPBA, CH_2Cl_2; (d) Pd/C, H_2, EtOH.

Table 1. Results of LAL Assay[a]

	LAL activity	
compd	EU/mL	ng equiv LPS/ mg product
1(*R/S) [OM-294-DP (*R*/*S*,*R*)]	874 ± 620	787 ± 558
1(*R) [OM-294-DP (*R*,*R*)]	1072 ± 205	965 ± 184
1(*S) [OM-294-DP (*S*,*R*)]	240 ± 196	216 ± 176
2(*R/S) [OM-294-MP (*R*/*S*,*R*)]	16238 ± 8974	14614 ± 8077
2(*R) [OM-294-MP (*R*,*R*)]	14257 ± 10324	12831 ± 9295
2(*S) [OM-294-MP (*S*,*R*)]	9320 ± 2462	8388 ± 2216

[a] Results are expressed in endotoxin units (EU) or as nanogram equivalent LPS per milligram of product, one EU corresponding here to 0.09 ng equiv LPS. Test compound concentration is 0.1 mg/mL. Average results from three independent experiments.

Table 2. Results of Pyrogenicity in Rabbits

	sum of increased temperature in three rabbits (°C)	
doses (mg/kg)	**1(*R/S)** [OM-294-DP (*R*/*S*,*R*)]	**2(*R/S)** [OM-294-MP (*R*/*S*,*R*)]
control (water)	0.80 (pass)	0.80 (pass)
0.001	0.60 (pass)	0.85 (pass)
0.01	0.75 (pass)	0.95 (pass)
0.1	1.35 (inconclusive)	3.65 (fail)

Furthermore, the results of Table 1 show that the stereochemistry of the carbon from the aspartic acid moiety (C-2b) does not seem to play a crucial role for the endotoxic potential.

Pyrogenicity. These in vitro results were confirmed by an in vivo pyrogenicity test for compounds OM-294-DP [**1(*R/S)**] and OM-294-MP [**2(*R/S)**] in the rabbit, since the pyrogenicity induced by the iv injection of OM-294-MP [**2(*R/S)**] was always higher than the pyrogenicity induced by OM-294-DP [**1(*R/S)**] (see Table 2). It should be noted that with doses up to 0.01 mg/kg both compounds were far below the allowed regulatory limit of defining whether a product is considered pyrogenic (according to the European Pharmacopoeia for the Assessment of Pyrogenicity in the Rabbit).

NO−Production by Murine Macrophages. A simple in vitro test for evaluating immunostimulating activity is the induction of NO synthase in macrophages, resulting in the production of the highly reactive NO radical. The cytotoxic activity of macrophages is due, in part, to the destructive action of NO on microbial DNA and membranes.[36] The ability of compound **1(*R/S)**, of the homogeneous stereoisomers **1(*S)** and **1(*R)**, of compound **2(*R/S)**, and of the homogeneous stereoisomers **2(*S)** and **2(*R)** to induce the production of NO by murine macrophages was determined, and the results are shown in Figure 4. The molecules of the series **1** (OM-294-DP) are powerful inducers of NO production, some activity being detected at the low dose of 0.01 μg/mL; this activity is comparable to the effect of the "parent" biological molecule OM-174. In contrast, only a faint activity could be detected for the compounds of the second series (OM-294-MP) at the dose of 0.2 μg/mL. The LPS control is also shown in Figure 4. Interestingly, the results of Figure 4 show that stereochemistry of the carbon from the aspartic acid moiety (C-2b) does not seem to play a discriminating role for NO production by murine macrophages.

Production of Interleukin-6 (IL-6) by Human PBMC. The production of IL-6 by human peripheral blood mononuclear cells (PBMC) is a further important in vitro test to screen the ability of new compounds to stimulate the immune system. IL-6 is a multifunctional cytokine that plays important roles in host defense, acute phase reactions, and hematopoiesis.[37] The agonistic ability of compounds **1** and **2** per se to induce (at 20 μg/mL) the production of IL-6 by human PBMC was determined, and the results are shown in Figure 5. Interestingly, compounds of the series **1** were unable to induce any production of IL-6, and some compounds of the series **2**—2(***R**) and **2(*R/S)**—were slightly active per se, when compared to the positive control LPS.

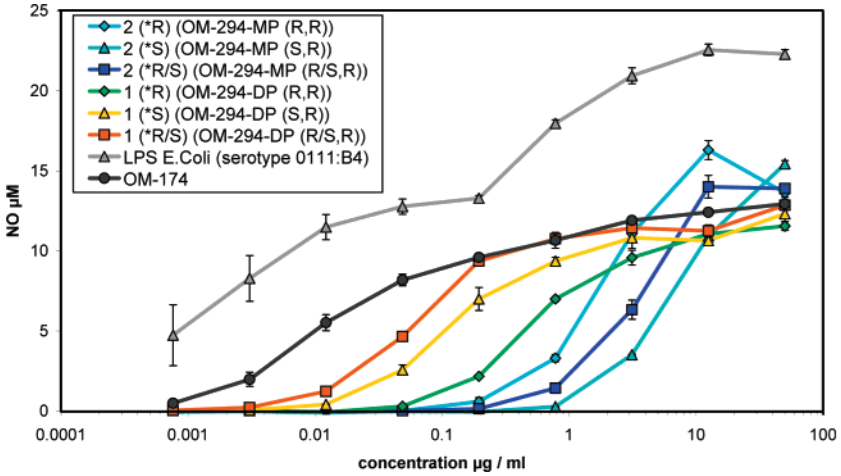
Finally, the ability of the compounds (at 0.02, 0.2, 2, and 20 μg/mL) to antagonize the LPS-induced (at 10 ng/mL) secretion of IL-6 by human PBMC was also determined (Figure 6). Very interestingly, compounds of the series **1** at 2 μg/mL, when added 90 min before LPS (or even concomitantly to LPS, not shown), were always able to almost completely abolish the LPS-induced production of IL-6, and this property was never observed with compounds of the series **2** at 2 μg/mL. Interestingly, when LPS was added before the compounds of the series **1** and **2**, no antagonistic effect could be detected any more. This may suggest that the compounds of the series **1** and LPS compete for the same receptor, probably TLR4. Additional experiments in vitro and in vivo will be necessary to verify if any of the compounds presented here will be worth developing clinically against inflammatory pathologies such as septic shock. Finally, the stereochemistry of the carbon from the aspartic acid moiety (C-2b) did not play any significant role in this antagonistic effect for any of the compounds tested.

Conclusion

Compounds of the series **1** (OM-294-DP) and **2** (OM-294-MP) represent the first members of a new class of lipid A mimics based on a pseudodipeptide backbone carrying only the essential functionalities of the parent lipid A structure (OM-174). These compounds possess very interesting partial immunological activities (either immunostimulating or immunomodulating), comparable to that of the parent lipid A, and are practically devoid of endotoxicity. Further experiments both in vitro and in vivo are now necessary to select from among the compounds described here a lead compound for clinical development.

Experimental Section

Methyl (*R*)-3-Benzyloxytetradecanoate (4). To a solution of methyl (*R*)-3-hydroxytetradecanoate **3** (10.0 g, 38.7 mmol) in anhydrous THF (150 mL) were added triethylamine (11.9 mL, 85.4 mmol) and chlorotrimethylsilane (9.8 mL, 77.2 mmol). The mixture was stirred at room temperature until all the starting material had



Compounds	EC_{50} (µg/mL)
2 (*R) (OM-294-MP (R,R))	1.85
2 (*S) (OM-294-MP (S,R))	6.86
2 (*R/S) (OM-294-MP (R/S,R))	3.53
1 (*R) (OM-294-DP (R,R))	0.55
1 (*S) (OM-294-DP (S,R))	0.15
1 (*R/S) (OM-294-DP (R/S,R))	0.083
LPS E. Coli (serotype 0111 :B4)	0.011
OM-174	0.025

Figure 4. NO production by murine macrophages.

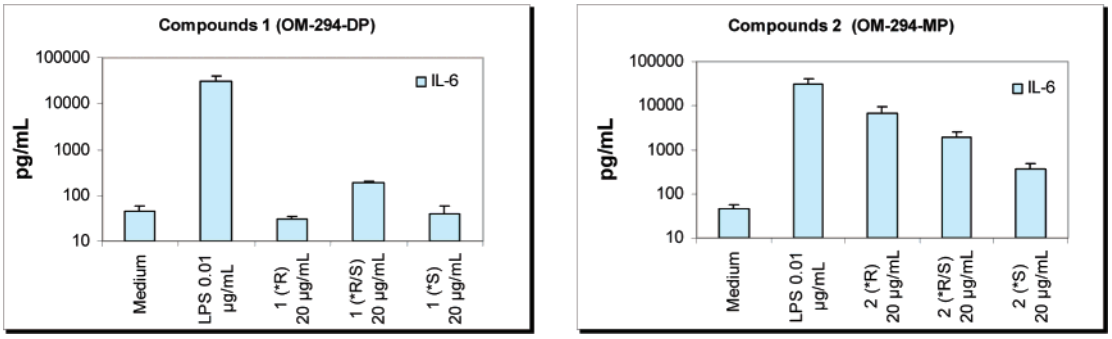


Figure 5. Production of IL-6 by human PBMC[a] by compounds of series **1** and **2**. Concentration of IL-6 produced (pg/mL) ± SEM ($n = 3$ independent experiments) at 20 μg/mL of the test compounds and at 0.01 μg/mL for LPS of *E. coli* (serotype 055:B5, Sigma, St. Louis, MO).

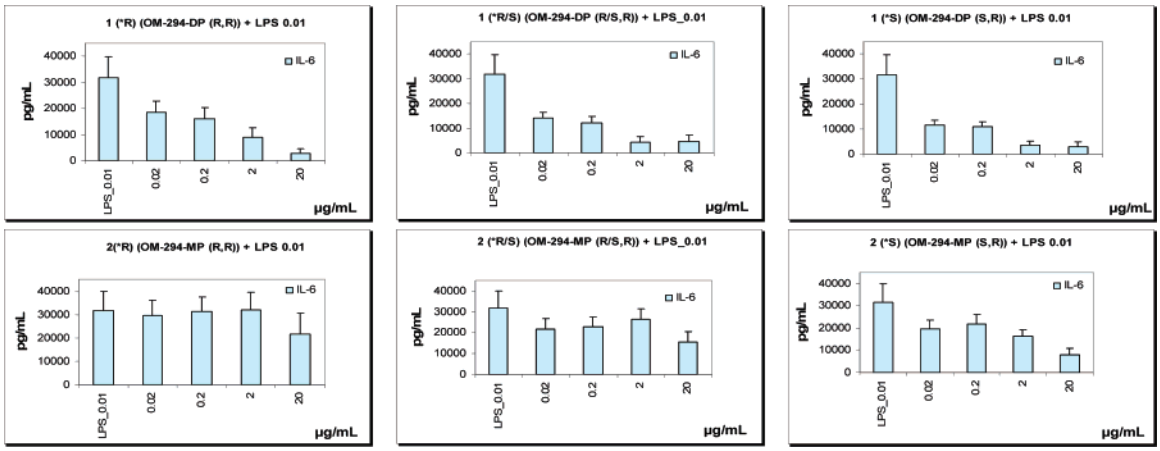


Figure 6. The antagonistic effect of compounds of the series **1** and **2** on LPS-induced IL-6 production by human PBMC. Concentration of IL-6 produced (pg/mL) ± SEM ($n = 3$ independent experiments) at 0.02, 0.2, 2, and 20 μg/mL of the test compounds with addition of 0.01 μg/mL of *E. coli* LPS (serotype 055:B5, Sigma, St. Louis, MO).

disappeared (17 h). CH_2Cl_2 (50 mL) was then added and the reaction mixture was cooled in an ice bath. A saturated solution of $NaHCO_3$ (5 mL) was added, followed by cold water (50 mL). The organic phase was separated and washed with water (100 mL). The aqueous phases were combined and washed with ether (2 × 100 mL); the combined organic phases were dried over $MgSO_4$ and concentrated, thus affording the crude trimethylsilyl ether of **3** (12.7 g). To an ice-cold solution of this crude silyl ether (12.7 g, 38.4 mmol) and benzaldehyde (4.7 mL, 46.2 mmol) in anhydrous CH_2Cl_2 (300 mL) was added trimethylsilyl triflate (0.7 mL, 3.87 mmol), and the mixture was stirred for 1 h at 0 °C. Triethylsilane (6.1 mL, 38.2 mmol) was then added and the mixture was allowed to warm to room temperature. After 14 h, the reaction mixture was diluted with ether (150 mL) and washed with a saturated solution of $NaHCO_3$ (200 mL) and then with water (100 mL). The organic phase was dried over $MgSO_4$ and the solvent was evaporated. The crude methyl (*R*)-3-benzyloxytetradecanoate **4**[20a,c] thus obtained (18.2 g, homogeneous by NMR) was used without further purification in the next step.

(*R*)-3-Benzyloxytetradecanoic Acid (5). To a solution of crude methyl (*R*)-3-benzyloxytetradecanoate **4** (18.2 g) in THF (200 mL) was added 1 N aqueous LiOH (200 mL, 0.2 mol) and the mixture was heated at 80 °C for 30 min and at 50 °C for 6 h. The mixture was neutralized with 1 N aqueous HCl and then extracted with ether (2 × 150 mL). The organic phases were combined, washed with water, dried over MgSO4, and concentrated. The residue was purified by flash column chromatography on silica gel (EtOAc/hexane, 1:20 → 1:3) which afforded pure **5** (10.4 g, 81% from **3**):

$[\alpha]_D$ −5 (*c* 1.2, $CHCl_3$) {lit. [24] $[\alpha]_D$ −4.4 (*c* 1.6, $CHCl_3$)}. ^{13}C NMR ($CDCl_3$, 90 MHz) δ 177.4, 138.2, 128.3, 127.8, 127.6, 75.8, 71.5, 39.6, 34.2, 31.9, 29.3−29.6, 25.1, 22.6, 14.1.

Benzyl (*R*)-3-Dodecanoyloxytetradecanoate (7). To a suspension of (*R*)-3-hydroxytetradecanoic acid (17.5 g, 72 mmol) in ethyl acetate (320 mL) were added benzyl bromide (25.6 mL, 0.22 mol), triethylamine (30 mL, 0.21 mol), and tetrabutylammonium iodide (13.3 g, 36 mmol). The mixture was stirred for 18 h at room temperature. The solvent was then evaporated, and the residue was taken up in ether (300 mL) and washed with saturated aqueous $NaHCO_3$ (250 mL) and then with water (2 × 250 mL). The organic phase was separated and dried over $MgSO_4$. The solvent was evaporated, thus affording crude benzyl (*R*)-3-hydroxytetradecanoate[22] **6** (26.0 g). Crude **6** (22.8 g, 68 mmol) was acylated with dodecanoyl chloride (16 mL, 69 mmol) in a mixture of pyridine (17 mL) and CH_2Cl_2 (360 mL) at 0 °C and then at room temperature for 18 h. The reaction mixture was poured into ice−water containing 5% aqueous $NaHCO_3$ and the organic phase was separated. The organic phase was washed with 1 N aqueous HCl and then with brine (50 mL) and water (50 mL). The organic phase was separated, dried over $MgSO_4$, and filtered. The solvent was evaporated and the residue was purified by flash chromatography on silica gel (hexane/EtOAc, 20:1) to give **7** (31.3 g, 96% for two steps) as a colorless syrup: $[\alpha]_D$ +1.8 (*c* 5.20, $CHCl_3$); ν_{max} (thin film)/cm^{-1} 2925, 2854, 1740, 1465, 1163, 749; ^1H NMR (250 MHz, $CDCl_3$) δ 7.35 (m, 5H, Ph), 5.21 (m, 1H, H-3), 5.18 (s, 2H, CH_2Ph), 2.63 (dd, 1H, *J* = 15.2, 7.1 Hz, H-2B), 2.55 (dd, 1H, *J* = 15.2, 5.6 Hz, H-2A), 2.20 (t, 2H, *J* = 7.4 Hz, 2 H-2′), 1.70−1.45 (br, 4H), 1.40−1.10 (br, 34H, 17 CH_2), 0.87 (m, 6H, 2 CH_3); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 172.8, 170.0, 135.6, 128.3, 128.1, 128.0, 70.0, 66.2, 39.1, 34.2, 33.9, 31.8, 29.5, 29.4, 29.3, 29.2, 29.0, 24.9, 24.8, 22.5, 13.9; MS-IS 539.5 $[M + Na]^+$, 517.5 $[M + H]^+$. Anal. ($C_{33}H_{56}O_4$) C, H.

(*R*)-3-Dodecanoyloxytetradecanoic Acid (8). To a solution of benzyl ester **7** (3 g, 5.8 mmol) in a mixture of ethyl acetate (30 mL) and ethanol (30 mL) was added 10% Pd on carbon (300 mg). The mixture was hydrogenated at room temperature under atmospheric pressure of hydrogen for 3 h. The catalyst was then removed by filtration and the filtrate was concentrated. The residue was recrystallized from pentane (−20 °C). The solvent was decanted and the solid washed rapidly with cold pentane. The mother liquors were further crystallized to give pure **8**[23,24] in two crops (2.13 g, 86%): mp 36−38 °C (lit.[23,24] oil); NMR spectra identical to the reported data.[24]

(2*R*)-5-Benzyloxycarbonylamino-2-*tert*-butyloxycarbonylaminopentan-1-ol (10). To a cold solution (−15 °C) of Boc-D-Orn-(Z)-OH (5.45 g, 14.9 mmol) in THF (60 mL) were added *N*-methylmorpholine (1.65 mL, 14.9 mmol) and isobutyl chloroformate (1.95 mL, 14.9 mmol). The mixture was stirred for 1 min at −15 °C and then a solution of $NaBH_4$ (1.70 g, 44.7 mmol) in H_2O (10 mL) was added (**CAUTION**: evolution of hydrogen). Stirring was continued for 10 min at −15 °C, and then H_2O (200 mL) was added to quench the reaction. The mixture was extracted with EtOAc (2 × 100 mL), and the organic layers were combined and washed with H_2O (50 mL) and brine (60 mL). The organic phase was separated and dried over $MgSO_4$. The solvent was evaporated and the residue was crystallized from EtOAc−hexane to give D-ornithinol derivative **10** (4.94 g, 94%) as a white solid: mp 47.5−48.0 °C; $[\alpha]_D$ +9.5 (*c* 4.0, $CDCl_3$); ν_{max} (KBr)/cm^{-1} 3343, 2978, 2945, 1697, 1530, 1267, 1172; ^1H NMR (360 MHz, $CDCl_3$) δ 7.31−7.27 (m, 5H, Ph), 5.22 (br, 1H, NH), 5.06 (s, 2H, CH_2Ph), 4.97 (br, 1H, NH), 3.66 (m, 2H, 2 H-1), 3.55 (m, 1H, H-2), 3.15 (m, 2H, 2 H-5), 1.54 (m, 4H, 2 CH_2), 1.40 (s, 9H, 3 CH_3); ^{13}C NMR (90 MHz, $CDCl_3$) δ 156.6, 156.3, 136.6, 128.5, 128.1, 79.6 (C), 66.6 (CH_2), 65.3 (CH_2), 52.4 (CH), 40.8 (CH_2), 28.7 (CH_2), 28.4 (CH_3), 26.6 (CH_2); MS-IS 391.5 $[M + K]^+$, 375.0 $[M + Na]^+$, 370.5 $[M + NH_4]^+$, 353.0 $[M + H]^+$. Anal. ($C_{18}H_{28}N_2O_5$) C, H, N.

(2*R*)-5-Benzyloxycarbonylamino-2-aminopentan-1-ol, Trifluoroacetic Salt (11). Compound **10** (6.32 g, 17.95 mmol) was dissolved in TFA (25 mL) and the solution was stirred at room temperature for 2.5 h. The solvent was then evaporated and the residue was purified by flash chromatography on silica gel (MeOH/CH_2Cl_2, 10:1) to give **11** (5.45 g, 83%) as a colorless syrup: R_f = 0.23 (MeOH/CH_2Cl_2, 10:1); ^1H NMR (250 MHz, CD_3OD) δ 7.40−7.20 (m, 5H, Ph), 5.00 (s, 2H, CH_2Ph), 3.75 (dd, 1H, *J* = 11.1, 3.8 Hz, H-1B), 3.55 (dd, 1H, *J* = 11.1, 6.2 Hz, H-1A), 3.15 (m, 3H, H-2, 2 H-5), 1.60 (m, 4H, 2 CH_2); ^{13}C NMR (62.9 MHz, CD_3OD) δ 162.3, 161.7, 161.2, 160.6, 157.5, 136.7, 128.0, 127.5, 127.3, 118.8, 114.1, 66.0, 60.6, 52.8, 26.0, 25.3.

(2*R*)-5-Benzyloxycarbonylamino-2-[(*R*)-3-benzyloxytetradecanoylamino]pentan-1-ol (12). To a cold solution (−15 °C) of (*R*)-3-benzyloxytetradecanoic acid **5** (5.27 g, 15.78 mmol) in THF (30 mL) were added *N*-methylmorpholine (1.89 mL, 15.78 mmol) and isobutyl chloroformate (2.21 mL, 15.78 mmol). The reaction mixture was stirred at −15 °C for 30 min. A solution of compound **11** (5.25 g, 14.34 mmol) in a mixture of THF (30 mL) and Et_3N (1.44 mL) was added to the reaction mixture. Stirring was continued for 16 h at room temperature. Water (30 mL) and EtOAc (60 mL) were then added, the organic phase was separated, and the aqueous phase was extracted with EtOAc (60 mL) once more. The organic layers were combined, washed with H_2O (30 mL) and brine (30 mL), and dried over $MgSO_4$. The solvent was evaporated and the residue was crystallized from EtOAc−hexane to give **12** (5.82 g, 69%) as a white solid: mp 117.5−118.0 °C; $[\alpha]_D$ +2 (*c* 1.30, $CHCl_3$); ν_{max} (KBr)/cm^{-1} 3298, 3090, 2921, 2851, 1690, 1642, 1539, 1264, 1027, 739, 697; ^1H NMR (360 MHz, $CDCl_3$) δ 7.35−7.25 (m, 10H, Ph), 6.55 (br d, 1H, *J* ∼ 7.5 Hz, N(2)−H), 5.06 (s, 2H, CH_2Ph), 4.98 (br, 1H, N(5)−H), 4.56 (d, 1H, *J* = 11.1 Hz, CH_2Ph), 4.45 (d, 1H, *J* = 11.1 Hz, CH_2Ph), 3.83 (m, 2H, H-2, H-3′), 3.50 (br dd, 1H, *J* ∼ 3.5, 11 Hz, H-1B), 3.43 (m, 1H, H-1A), 3.11 (m, 2H, 2 H-5), 2.80 (br, 1H, OH), 2.44 (dd, 1H, *J* = 4, 14.6 Hz, H-2′B), 2.37 (dd, 1H, *J* = 7.6, 14.7 Hz, H-2′A), 1.68−1.42 (m, 6H, 3 CH_2), 1.33−1.25 (m, 18H, 9 CH_2), 0.88 (t, 3H, CH_3); ^{13}C NMR (90 MHz, $CDCl_3$) δ 172.2, 156.5, 138.2, 136.6, 128.5−127.9, 76.8, 71.4, 66.6, 65.5, 51.5, 41.5, 40.7, 33.9, 31.9, 29.6−29.3, 28.1, 26.5, 25.1, 22.6, 14.1; MS-IS 591.5 $[M + Na]^+$, 569.5 $[M + H]^+$. Anal. ($C_{34}H_{52}N_2O_5$) C, H, N.

(2*R*)-5-Amino-2-[(*R*)-3-benzyloxytetradecanoylamino]pentan-1-ol (13). A solution of compound **12** (3.00 g; 5.27 mmol) in a mixture of EtOH (300 mL) and Et_3N (6 mL) was hydrogenated for 2 h in the presence of 20% Pd on carbon (150 mg) at room temperature under H_2 (atmospheric pressure). The catalyst was removed by filtration and the filtrate was concentrated in vacuo to give amine **13** as a white solid that was used directly in the next step without purification: R_f = 0.20 (CH_2Cl_2/MeOH/Et_3N 5:1:0.5); mp 47−48 °C; $[\alpha]_D$ +9.5 (*c* 1.05, $CHCl_3$); ν_{max} (KBr)/cm^{-1} 3289, 3089, 2920, 2852, 1638, 1554, 1072, 740, 696; ^1H NMR (360 MHz, $CDCl_3$) δ 7.33−7.24 (m, 5H, Ph), 6.77 (br, 1H, NH), 4.51 (AB, 2H, *J* = 11.3 Hz, CH_2Ph), 3.84 (m, 2H, H-2, H-3′), 3.50 (m, 2H, 2 H-1), 2.89 (br, 3H, 2 H-5, OH), 2.58 (br, 2H, NH_2), 2.43−2.33 (ABX, 2H, 2 H-2′), 1.68−1.4 (m, 6H, 3 CH_2), 1.33−1.25 (m, 18H, 9 CH_2), 0.88 (t, 3H, CH_3); ^{13}C NMR (90 MHz, $CDCl_3$) δ 172.0, 138.2, 128.4, 127.9, 127.7, 76.8, 71.5, 64.9, 51.5, 41.6, 41.3, 33.9, 31.9, 29.6, 29.3, 28.7, 28.1, 25.1, 22.6, 14.1; MS-IS 457.0 $[M + Na]^+$, 435.0 $[M + H]^+$.

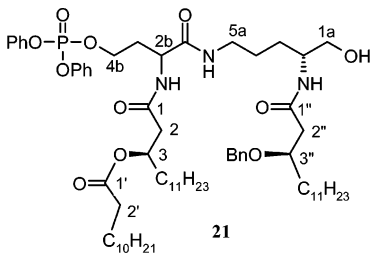
(2*R*)-5-(Benzyloxycarbonylamino)-2-[(*R*)-3-benzyloxytetradecanoylamino]pentan-1-ol Benzyloxymethyl Ether (14). To a stirred solution of **12** (2.05 g, 3.60 mmol) in CH_2Cl_2 (40 mL) at room temperature were added successively benzyl chloromethyl ether (60%, 1.25 mL, 5.41 mmol) and *N*,*N*-diisopropylethylamine (942 μL, 5.41 mmol). After having been stirred overnight at room temperature, the mixture was concentrated and the residue was purified by flash chromatography on silica gel (light petroleum/EtOAc, 2:1) to give compound **14** (2.28 g, 92%) as a white solid: R_f = 0.70 (light petroleum/EtOAc, 1:3); mp 97−100 °C; $[\alpha]_D$ −4 (*c* 1.08, $CHCl_3$); ν_{max} (KBr)/cm^{-1} 3297, 2921, 2851, 1688, 1642, 1547, 1264, 695; ^1H NMR (250 MHz, $CDCl_3$) δ 7.40−7.20 (m, 5H, Ph), 6.46 (d, 1H, *J* = 8.8 Hz, NH), 5.08 (s, 2H, CH_2Ph), 4.95 (br, 1H, NH), 4.65−4.45 (2s and AB, 6H, 2 CH_2Ph, OCH_2O), 4.08 (m, 1H, H-3′), 3.81 (m, 1H, H-2), 3.56 (dd, 1H, *J* = 3.8, 10 Hz, H-1B), 3.43 (dd, 1H, *J* = 4.1, 10 Hz, H-1A), 3.15 (m, 2H, 2 H-5),

2.45−2.3 (ABX, 2H, 2 H-2′), 1.68−1.42 (m, 6H, 3 CH_2), 1.40−1.15 (m, 18H, 9 CH_2), 0.88 (t, 3H, CH_3); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 170.8, 156.3, 138.2, 137.6, 136.6, 128.4, 128.3, 128.2, 127.9, 127.6, 127.5, 94.8, 76.6, 71.2, 69.6, 69.5, 66.4, 48.3, 41.4, 40.7, 33.8, 31.8, 29.6, 29.5, 29.2, 29.0, 26.3, 25.0, 22.6, 14.0; MS-IS 689.5 $[M + H]^+$. Anal. ($C_{42}H_{61}N_2O_6$) C, H, N.

(2*R*)-5-Amino-2-[(*R*)-3-benzyloxytetradecanoylamino]pentan-1-ol Benzyloxymethyl Ether (15). A solution of compound **14** (2.00 g; 2.90 mmol) in a mixture of EtOH (220 mL) and Et_3N (4 mL) was hydrogenated for 3 h in the presence of 20% $Pd(OH)_2$ on carbon (200 mg) at room temperature under H_2 (atmospheric pressure). The catalyst was removed by filtration and the filtrate was concentrated in vacuo. The white solid was dried under vacuum to give amine **15** (1.58 g; 98%). $[\alpha]_D$ −1 (*c* 1.20; $CHCl_3$); ν_{max} (KBr)/cm^{-1} 3313, 2919, 2851, 1635, 1542, 1048, 695; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.45−7.21 (m, 10H, Ph), 6.52 (d, 1H, J = 8.8 Hz, NH), 4.80−4.45 (m, 6H, 2 CH_2Ph, OCH_2O), 4.10 (m, 1H, H-3′), 3.83 (m, 1H, H-2), 3.62 (dd, 1H, J = 3.9, 10.0 Hz, H-1B), 3.47 (dd, 1H, J = 4.4, 10.0 Hz, H-1A), 2.65 (t, 2H, J = 6.4 Hz, 2 H-5), 2.48−2.32 (ABX, 2H, 2 H-2′), 1.80−1.40 (m, 8H, 3 CH_2, NH_2), 1.40−1.20 (m, 18H, 9 CH_2), 0.88 (t, 3H, J = 6.3 Hz, CH_3); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 170.8, 138.2, 137.6, 128.3, 127.6, 94.8, 76.7, 71.2, 69.6, 69.4, 48.4, 41.8, 41.4, 33.8, 31.8, 29.8, 29.5−29.0, 25.1, 22.6, 14.0; MS-IS 555.5 $[M + H]^+$.

***N*-*tert*-Butoxycarbonyl *O*-Bis(phenyloxy)phosphoryl DL-Homoserine Benzyl Ester (17).** Commercially available DL-homoserine hydrobromide (2.0 g, 16.78 mmol) was dissolved in H_2O (20 mL). To this solution were added successively 1 N aqueous NaOH (16.8 mL) and solid cesium carbonate (3.00 g, 9.23 mmol). After having been stirred for 5 min, the solution was cooled in an ice−water bath. Dioxane (60 mL) and di-*tert*-butyl dicarbonate (5.44 g, 25.17 mmol) were then added. The reaction mixture was stirred at 0 °C for 1 h and then at room temperature for 5 h. The solvent was removed in vacuo and the residue was dried by coevaporation with DMF (20 mL). DMF (60 mL) and benzyl bromide (4.50 mL, 20.13 mmol) were then added to the crude cesium salt, which resulted in the formation of a white precipitate of cesium bromide. The mixture was stirred for 16 h and the solvent was then removed in vacuo. The residue was extracted with EtOAc (2 × 20 mL). The organic layers were combined, washed successively with H_2O (20 mL) and brine (20 mL), and dried over $MgSO_4$. The solvent was evaporated and the residue was dried under high vacuum to give crude *N*-*tert*-butoxycarbonyl DL-homoserine benzyl ester **16**. To a solution of this crude material in CH_2Cl_2 (60 mL) was added DMAP (4.11 g, 33.56 mmol), and the reaction mixture was stirred for 10 min. Pyridine (12 mL) and diphenyl chlorophosphate (6.95 mL, 33.56 mmol) were then added and the solution was stirred at room temperature for 18 h. The mixture was then washed successively with 1 N aqueous HCl (20 mL), H_2O (30 mL), and brine (30 mL). The organic layer was separated and dried over $MgSO_4$. The solvent was evaporated and the residue was purified by flash chromatography on silica gel (hexane/EtOAc, 4:1) followed by crystallization to give **17** as a white solid (7.49 g, yield: 82% from DL-homoserine hydrobromide): R_f = 0.13 (hexane/EtOAc, 4:1); mp 63.5−64.0 °C; ν_{max} (KBr)/cm^{-1} 3304, 2974, 1740, 1702, 1524, 1487, 1275, 1184, 1159, 964; ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.34−7.28 (m, 9H, Ph), 7.25−7.15 (m, 6H, Ph), 5.19 (br d, 1H, NH), 5.13 (∼s, 2H, CH_2Ph), 4.42 (m, 1H, H-2), 4.33 (∼q, 2H, 2 H-4), 2.26 (m, 1H, H-3B), 2.14 (m, 1H, H-3A), 1.42 (s, 9H, *t*-Bu); ^{13}C NMR (90 MHz, $CDCl_3$) δ 171.6, 155.2, 150.4 (d), 135.2, 129.8, 128.6, 128.4, 128.3, 125.4, 120.0 (d), 80.1, 67.3, 65.3 (d), 50.7, 32.7 (d), 28.2; ^{31}P NMR (121.5 MHz) δ −11.42 ($^3J_{P,\,H}$ = 7.4 Hz); MS-FAB 542.2 $[M + H]^+$; HRMS-FAB calcd for $C_{28}H_{32}NO_8P$ $[M + H]^+$ 542.1944, found 542.1956.

***O*-Bis(phenyloxy)phosphoryl DL-Homoserine Benzyl Ester, Trifluoroacetic Salt (18).** Compound **17** (7.88 g, 14.56 mmol) was dissolved in TFA (15 mL) and the solution was stirred at room temperature for 2.5 h. The solvent was then removed under high vacuum. Purification of the residue by flash chromatography on silica gel (MeOH/CH_2Cl_2, 10:1) followed by crystallization gave **18** (7.17 g, 89%) as a white solid: R_f = 0.18 (MeOH/CH_2Cl_2, 10:1); mp 73.0−73.5 °C; ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.30−7.25 (m, 9H, Ph), 7.23−7.05 (m, 6H, Ph), 5.10 (s, 2H, CH_2Ph), 4.38 (m, 2H, 2 H-4), 4.05 (t, 1H, J = 6.2 Hz, H-2), 2.34 (br, 2H, 2 H-3); ^{13}C NMR (90 MHz, $CDCl_3$) δ 168.6, 150.1 (m), 134.3, 129.9, 128.7, 128.6, 128.5, 125.6, 120.0 (2 d), 68.5, 64.5 (d, J = 6 Hz), 49.7, 30.8 (d, J = 6.6 Hz).



21

Figure 7. Numbering scheme of **21**.

***N*-[(*R*)-3-Dodecanoyloxytetradecanoyl] *O*-Bis(phenyloxy)-phosphoryl DL-Homoserine Benzyl Ester (19).** To a stirred solution of (*R*)-3-dodecanoyloxytetradecanoic acid **8** (4.28 g, 10.07 mmol) in THF (30 mL) at −15 °C were added successively *N*-methylmorpholine (1.11 mL, 10.07 mmol) and isobutyl chloroformate (1.31 mL, 10.07 mmol). Stirring was continued for 30 min at −15 °C. A solution of **18** (5.73 g, 10.07 mmol) in a mixture of THF (30 mL) and Et_3N (5 mL) was then added to the reaction mixture. After stirring overnight at room temperature, the solvent was removed in vacuo and H_2O (20 mL) was added to the residue. The mixture was then extracted with EtOAc (2 × 30 mL), and the organic phases were combined, washed successively with H_2O (20 mL) and brine (20 mL), and dried over $MgSO_4$. The solvent was evaporated and the residue was purified by flash chromatography on silica gel (hexane/EtOAc, 2:1) to give **19** (7.46 g, 87%) as a white solid: R_f = 0.29 (hexane/EtOAc, 2:1); mp 31.0−32.1 °C; ν_{max} (KBr)/cm^{-1} 3305, 3066, 2922, 2852, 1731, 1681, 1537, 1489, 1288, 1190, 1025, 954, 754, 689; ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.27−7.36 (m, 9H, Ph), 7.16−7.20 (m, 6H, Ph), 6.62 (2d, 1H, J ∼ 8 Hz, NH), 5.09−5.20 (m, 3H, H-3′, CH_2Ph), 4.68 (m, 1H, H-2), 4.31 (m, 2H, 2 H-4), 2.39−2.51 (m, 2H, 2 H-2′), 2.20−2.34 (m, 4H, 2 CH_2), 1.58 (m, 4H, 2 CH_2), 1.25 (m, 34H, 17 CH_2), 0.88 (m, 6H, 2 CH_3); ^{13}C NMR (90 MHz, $CDCl_3$) δ 173.4, 171.2, 169.7, 150.4 (d), 135.1, 129.8, 128.3−128.8, 125.4, 120.0 (d), 71.0 (2 signals), 67.5, 65.3 (2 signals), 49.5 (2 signals), 41.5 and 41.3, 34.4, 34.2 and 34.0, 32.3, 32.2, 31.9, 29.2−29.6, 25.2 and 25.0, 22.7, 14.1; ^{31}P NMR (121.5 MHz) δ −11.36, −11.37 ($^3J_{P,\,H}$ = 7.1 Hz); MS-FAB 850.4 $[M + H]^+$; HRMS-FAB calcd for $C_{49}H_{72}NO_9P$ $[M + H]^+$ 850.5023, found 850.5024.

***O*-Bis(phenyloxy)phosphoryl *N*-[(*R*)-3-Dodecanoyloxytetradecanoyl] DL-Homoserine (20).** 20% Pd−C (40 mg) was added to a solution of **19** (85 mg, 0.1 mmol) in EtOH (20 mL) in a three-necked flask. (The reaction is faster if the catalyst is activated with hydrogen prior to the addition of the substrate.) Air was removed under vacuum, then the flask was charged with hydrogen (atmospheric pressure). The reaction mixture was stirred at room temperature for 3 h, the catalyst was removed by filtration, and the filtrate was concentrated to give labile acid **20** as a colorless syrup that was used in the next step without purification: ^{13}C NMR (90 MHz, $CDCl_3$) δ 173.9, 173.7, 170.7, 150.2, 129.9, 125.7, 120.0, 71.1, 65.9, 49.7, 41.2, 34.2, 34.1, 31.9, 29.1−29.6, 25.2, 25.0, 22.6, 14.0.

***O*-Bis(phenyloxy)phosphoryl *N*α-[(*R*)-3-Dodecanoyloxytetradecanoyl] DL-Homoserine, *N*-{(4*R*)-5-Hydroxy-4-[(*R*)-3-benzyloxytetradecanoylamino]pentyl}amide (21**,** Figure 7). IIDQ (36.1 mg, 0.12 mmol) was added to a solution of crude acid **20** (67 mg, 0.10 mmol) in CH_2Cl_2 (10 mL). After stirring for 15 min, a solution of amine **13** (53 mg, 0.10 mmol) in CH_2Cl_2 (5 mL) was introduced and the reaction mixture was stirred overnight. The solution was concentrated and the residue was purified by flash chromatography on silica gel (CH_2Cl_2/acetone, 5:2) to give amide **21** (66 mg, 56%) as a colorless syrup: R_f = 0.23 (CH_2Cl_2/acetone, 5:2); ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.14−7.33 (m, 15H, Ph), 7.00 (br, 1H, NH),

6.90 (d, 0.5H, J = 7.5 Hz, NH), 6.79 (d, 0.5H, J = 7.4 Hz, NH), 6.67 (d, 1H, J = 7.9 Hz, NH), 5.14 (m, 1H, H-3), 4.5 (m, 3H, H-2b, CH_2Ph), 4.29 (m, 2H, 2 H-4b), 3.83 (m, 2H, H-2a, H-3"), 3.41 (br m, 2H, 2 H-1a), 3.19 (m, 2H, 2 H-5a), 2.14−2.41 (3 m, 8H, 4 CH_2), 1.46−1.54 (br m, 8H, 4 CH_2), 1.22 (m, 54H, 27 CH_2), 0.85 (m, 9H, 3 CH_3); ^{13}C NMR (90 MHz, $CDCl_3$; two values given when two signals are observed for diastereomeric species) δ 173.5, 172.0, 170.5/170.4, 170.0/169.9, 150.2 (d), 138.4/138.3, 129.9, 128.3, 127.8, 127.6, 125.6, 120.05(d)/119.9(d), 76.6, 71.4, 71.0, 66.2/66.1, 64.9, 51.4/51.2, 50.2/50.1, 41.6/41.5, 39.3, 34.4/34.0, 32.9 (br), 31.8, 29.1−29.5, 28.4/28.2, 24.9−25.4, 22.6, 14.0; ^{31}P NMR (121.5 MHz) δ −11.2, −11.3 ($^3J_{P,\,H}$ = 8.5 Hz); MS-FAB 1176.7 $[M + H]^+$; HRMS-FAB calcd for $C_{68}H_{110}N_3O_{11}P$ $[M + H]^+$ 1176.7956, found 1176.7933.

***O*-Bis(phenyloxy)phosphoryl *N*-[(*R*)-3-Dodecanoyloxytetradecanoyl] DL-Homoserine, *N*-{(4*R*)-5-Bis(benzyloxy)phosphoryloxy-4-[(*R*)-3-benzyloxytetradecanoylamino]pentyl}amide (22).** To a stirred solution of amide **21** (300 mg, 0.26 mmol) and 1*H*-tetrazole (64 mg, 0.82 mmol) in THF (10 mL) at room temperature was added dibenzyl diethylphosphoramidite (85%, 230 mg, 0.62 mmol). Stirring was continued at room temperature for 30 min and the solution was then cooled to −20 °C. A solution of *m*CPBA (80−90%, 74 mg, 0.36 mmol) in CH_2Cl_2 (7 mL) was added and the solution was stirred for 20 min at room temperature. Aqueous sodium thiosulfate (10%, 6 mL) was added, the mixture was stirred for 10 min and then diluted with ether (30 mL), and the organic phase was separated. The organic layer was washed successively with 10% aqueous $Na_2S_2O_3$ solution (5×), saturated aqueous $NaHCO_3$ (2×), N HCl (1×). The organic phase was dried over $MgSO_4$ and the solvent removed in vacuo. Flash chromatography of the residue on silica gel (CH_2Cl_2/acetone, 10:3) provided compound **22** (242 mg, 65%) as a colorless oil: R_f = 0.64 (CH_2Cl_2/acetone 5:2); ν_{max} (thin film)/cm^{-1} 3277, 2921, 2851, 1793, 1737, 1657, 1537, 1490, 1224, 1090, 1025, 916, 745, 695; ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.13−7.31 (m, 25H, Ph), 6.86 (br d, 1H, NH), 6.73 (d, 0.5H, J = 7.5 Hz, NH), 6.68 (d, 0.5H, J = 8.5 Hz, NH), 6.64 (d, 1H, J = 8.2 Hz, NH), 5.17 (2 qt, 1H, H-3), 4.96 (m, 4H, 2 × POCH_2Ph), 4.52 (m, 1H, H-2b), 4.47 (s, 2H, CH_2Ph), 4.30 (br q, 2H, 2 H-4b), 3.8−4.0 (3 m, 4H), 3.05−3.25 (m, 2H), 2.15−2.41 (several m, 8H, 4 CH_2), 1.4−1.56 (m, 8H) and 1.22 (m, 54H) (31 CH_2), 0.85 (m, 9H, 3 CH_3); ^{13}C NMR (90 MHz, $CDCl_3$) δ 173.5/173.4, 173.1, 170.5/170.4, 169.9/169.7 (4 C═O), 150.4, 138.5, 135.7/135.6, 127.5−129.8, 125.5, 120.05 (d)/119.95 (d), 76.5, 71.2/71.0, 69.5/69.45, 68.7/68.6, 66.2 (d)/66.05 (d), 50.0/49.9, 48.5/48.45, 41.7/41.4, 39.0, 34.0−34.4, 32.9−33.1, 31.8, 29.1−29.6, 27.7, 24.95−25.2, 22.6, 14.0; ^{31}P NMR (121.5 MHz) δ −0.03 (^3J = 8 Hz, POBn), −11.00, −11.12 (^3J = 8 Hz, POPh); MS-IS 1436.5 $[M + H]^+$.

***O*-Bis(phenyloxy)phosphoryl *N*-[(*R*)-3-Dodecanoyloxytetradecanoyl] DL-Homoserine, *N*-{(4*R*)-5-Dihydroxyphosphoryloxy-4-[(*R*)-3-hydroxytetradecanoylamino]pentyl}amide (23).** Pd−C (20%, 40 mg) was added to a solution of **22** (160 mg, 0.11 mmol) in EtOH (20 mL) in a three-necked flask. Air was removed under vacuum and then the flask was charged with hydrogen (atmospheric pressure). Stirring was continued until no more starting material could be detected on TLC ($CHCl_3$/MeOH/H_2O, 6:4:0.6, new product has R_f = 0.63). The catalyst was removed by filtration, the filtrate was concentrated in vacuo, and the residual product dried under vacuum to give homogeneous **23** (100 mg; 78%): ^{13}C NMR (90 MHz, $CDCl_3$, after addition of Et_3N) δ 173.6, 173.5, 173.1, 171.2, 170.7, 170.4, 151.6, 150.3, 150.2, 129.9, 129.5, 125.6, 124.4, 120.2, 120.2, 120.1, 120.0, 120.0, 71.1, 69.2, 67.8 (br), 66.3 (m), 66.0, 58.4 (Et_3NH^+), 50.1, 49.3, 43.3 (br), 41.4, 41.3, 39.0, 37.3, 34.5, 34.3, 32.7 (br), 31.9, 29.2−29.7, 27.5, 25.0−25.6, 22.7, 18.3 (Et_3-NH^+), 14.1; ^{31}P NMR (121.5 MHz) δ 0.56 (POH), −10.77, −11.70 (POPh); MS-FAB 1188.7 $[M + Na]^+$; HRMS-FAB calcd for $C_{61}H_{105}N_3O_{14}P_2$ $[M + Na]^+$ 1188.6969, found 1188.6959.

***O*-Dihydroxyphosphoryl *N*-[(*R*)-3-Dodecanoyloxytetradecanoyl] DL-Homoserine, *N*-{(4*R*)-5-Dihydroxyphosphoryloxy-4-[(*R*)-3-hydroxytetradecanoylamino]pentyl}amide [1(*R/S)].** PtO_2 (60 mg) was added to a solution of **23** (100 mg, 86 μmol) in EtOH (20 mL) in a three-necked flask. Air was removed under vacuum and then the flask was charged with hydrogen (atmospheric pressure). The reaction mixture was stirred until no more starting material could be detected (∼ 72 h). The catalyst was removed by filtration, and the filtrate was concentrated to give **1(*R/S)** (67 mg, 77%) as a white glass. The product was dissolved in 1:1 (v/v) H_2O/*i*PrOH containing 0.1% Et_3N (pH 8−9); 2 M ammonium bicarbonate was then added to reach a 25 mM final concentration. The product was purified from this solution by RP HPLC [Bondapak gel C_{18}, 300 Å, 15−20 μm; column size 40 × 200 mm; solvent A, 1:9 (v/v) H_2O/*i*PrOH containing ammonium bicarbonate (50 mM); solvent B, 8:2 (v/v) H_2O/*i*PrOH containing ammonium bicarbonate (50 mM); 10 min 40% B; 10 min 40% → 80% B; 30 min 80% B; t_R = 21 min; UV (210 nm) detection]: R_f = 0.16 ($CHCl_3$/MeOH/H_2O, 6:4:0.6); ν_{max} (KBr)/cm^{-1} 3297, 2955, 2921, 2851, 1731, 1651, 1549, 1469, 1172, 1062; ^{13}C NMR (90 MHz, $CDCl_3$, broad signals) selected signals: δ 71.2, 69.3, 67.9, 63.5, 50.7, 49.6, 43.2, 41.4, 39.3, 37.2, 33.8−34.5, 32.8, 31.9, 29.1−29.8, 28.0, 27.5, 24.9−25.8, 22.7, 14.0; ^{31}P NMR (121.5 MHz) δ 0.98, 0.21. MS-FAB 1036.6 $[M + Na]^+$; 1058.5 $[M - H + 2Na]^+$; 1080.6 $[M - 2H + 3Na]^+$; HRMS-FAB calcd for $C_{49}H_{97}N_3O_{14}P_2$ $[M + Na]^+$ 1036.6344, found 1036.6347; HRMS-FAB calcd for $C_{49}H_{97}N_3O_{14}P_2$ $[M - H + 2Na]^+$ 1058.6163, found 1058.6158.

***O*-Bis(phenyloxy)phosphoryl *N*-[(*R*)-3-Dodecanoyloxytetradecanoyl] DL-Homoserine, *N*-{(4*R*)-5-Hydroxy-4-[(*R*)-3-hydroxytetradecanoylamino]pentyl}amide (24).** 20% Pd−C (40 mg) was added to a solution of **21** (150 mg, 0.13 mmol) in a mixture of EtOH (20 mL) and AcOH (0.6 mL) in a three-necked flask. Air was removed under vacuum and the flask was charged with hydrogen (atmospheric pressure). The mixture was stirred for 16 h at room temperature. The catalyst was removed by filtration and the filtrate was concentrated. Purification of the residue by flash chromatography on silica gel (CH_2Cl_2/acetone, 5:4) gave compound **24** (104 mg, 74%) as a glassy solid: R_f = 0.24 (CH_2Cl_2/acetone, 5:4); mp 67−68 °C;^{1}H NMR (360 MHz, $CDCl_3$) δ 7.15−7.35 (m, 10H, Ph), 7.03 (d, 0.5H, J = 7.3 Hz, NH), 6.91 (d, 0.5H, J = 7.1 Hz, NH), 6.79 (d, 1H, J = 8.0 Hz, NH), 5.16 (m, 1H, H-3), 4.50 (m, 1H, H-2b), 4.29 (m, 2H, 2 H-4b), 3.89 (m, 2H, H-2a, H-3″), 3.4−3.6 (m, 2H), 3.20 (m, 2H), 2.10−2.42 (m, 8H, 4 CH_2), 1.38−1.54 (m, 8H) and 1.22 (m, 54H) (31 CH_2), 0.85 (m, 9H, 3 CH_3); ^{13}C NMR (90 MHz, $CDCl_3$) δ 173.6, 173.2, 170.7/170.6, 170.3/170.1, 150.3, 129.9, 125.7, 120.1/120.0, 120.0/119.9, 71.1/71.0, 68.8, 66.2/66.1, 64.7, 51.4, 50.3/50.1, 43.2/43.1, 41.7/41.6, 39.3, 37.3, 34.4, 32.8, 31.9, 29.1−29.6, 28.1, 25.0−25.6, 22.6, 14.0; ^{31}P NMR (121.5 MHz) δ −11.28, −11.40 ($^3J_{P,\,H}$ = 7.8 Hz); MS-FAB 1086.8 $[M + H]^+$; HRMS-FAB calcd for $C_{61}H_{104}N_3O_{11}P$ $[M + H]^+$ 1086.7568, found 1086.7529.

***O*-Dihydroxyphosphoryl *N*-[(*R*)-3-Dodecanoyloxytetradecanoyl] DL-Homoserine, *N*-{(4*R*)-5-Hydroxy-4-[(*R*)-3-hydroxytetradecanoylamino]pentyl}amide (2(*R/S)).** PtO_2 (30 mg) was added to a solution of **24** (90 mg, 0.08 mmol) in EtOH (30 mL) in a three-necked flask. Air was removed under vacuum and then the flask was charged with hydrogen (atmospheric pressure). The reaction mixture was stirred until no more starting material could be detected by TLC ($CHCl_3$/MeOH/H_2O, 6:4:0.6, new product has R_f = 0.50). The catalyst was removed by filtration and the filtrate was concentrated to give **2(*R/S)** (60 mg, 78%) as a white solid. The product was dissolved in *i*PrOH/H_2O (1:1, v/v, ∼3 mg/mL) and the pH was adjusted to pH 8−9 by addition of Et_3N. A sample from this solution was purified by preparative HPLC (conditions: see preparation of **1(*R/S)**; t_R = 25 min): ^{13}C NMR (90 MHz, $CDCl_3$ + a drop of CD_3OD) δ 173.6, 173.3, 171.3, 170.4, 71.1, 68.8, 64.2, 62.6, 51.0, 50.2, 43.5, 41.5, 39.2, 37.3, 34.5, 33.7, 31.8, 29.2−29.8, 28.0, 25.0−25.8, 22.7, 14.1; ^{31}P NMR (121.5 MHz) δ 0.78 ($^3J_{P,\,H}$ = 7.8 Hz); MS-FAB 956.5 $[M + Na]^+$, 835.7 $[M - H_3PO_4]$; HRMS-FAB calcd for $C_{49}H_{96}N_3O_{11}P$ $[M + Na]^+$ 956.6680, found 956.6674.

***N*-[(*R*)-3-Dodecanoyloxytetradecanoyl]-D-aspartic Acid, β-Benzyl Ester (25).** To a stirred solution of (*R*)-3-dodecanoyloxytetradecanoic acid **8** (3.35 g, 7.85 mmol) in THF (25 mL) at −15 °C were added successively *N*-methylmorpholine (0.86 mL, 7.85

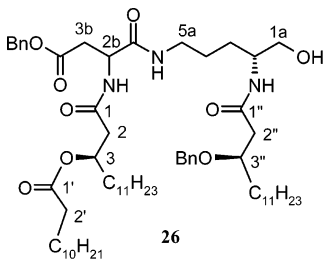


Figure 8. Numbering scheme of **26**.

mmol) and isobutyl chloroformate (1.02 mL, 7.85 mmol). After 30 min at −15 °C, a solution of H-D-Asp(OBn)-OH (Senn Chemicals) (1.75 g, 7.85 mmol) in 3.5:1 (v/v) CH_3CN/H_2O (85 mL) and Et_3N (3.7 mL) were added to the reaction mixture. After having been stirred overnight at room temperature, the mixture was partially concentrated and the residual aqueous phase was cooled to 0°C and acidified with 10% aqueous citric acid (pH = 3). The mixture was then extracted with EtOAc (2 × 30 mL), and the organic phases were combined and dried over $MgSO_4$. The solvent was evaporated and the residue was purified by flash chromatography on silica gel (light petroleum/EtOAc, 2:1 containing 2% acetic acid) to give **25** (4.00 g, 81%) as a white solid: R_f = 0.42 (light petroleum/EtOAc, 1:1 containing 2% acetic acid); mp 67−69 °C; $[\alpha]_D$ −19 (*c* 1.20, $CHCl_3$); ν_{max} (KBr)/cm^{-1} 3333, 3080, 1736, 1702, 1649, 1548, 1170; ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.90 (br, 1H, COOH), 7.30−7.50 (m, 5H, Ph), 6.89 (d, 1H, *J* = 7.8 Hz, NH), 5.10−5.20 (m and s, 3H, H-3, C*H*$_2$Ph), 4.87 (dt, 1H, *J* = 4.5, 4.5, 8 Hz, H-2), 3.09 (dd, 1H, *J* = 4.4, 17.6 Hz, H-3B), 2.90 (dd, 1H, *J* = 4.5, 17.6 Hz, H-3A), 2.48 (∼d, 2H, *J* = 6.3 Hz, 2 H-2′), 2.28 (t, 2H, *J* = 7.5 Hz, 2 H-2″), 1.60 (m, 4H) and 1.10−1.40 (m, 34H, 19 CH_2), 0.87 (t, 6H, 2 CH_3); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 174.1, 173.5, 171.0, 170.2, 135.2, 128.6, 128.4, 128.2, 125.8, 70.9, 66.9, 48.4, 41.2, 35.8, 34.4, 34.0, 31.8, 29.1−29.6, 25.2, 24.9, 22.6, 14.1; MS-IS 655.0 $[M + Na]^+$, 633.0 $[M + H]^+$. Anal. ($C_{37}H_{61}NO_7$) C, H, N.

***N*-[(*R*)-3-Dodecanoyloxytetradecanoyl]-D-Aspartic Acid, α-*N*-{(4*R*)-5-Hydroxy-4-[(*R*)-3-benzyloxytetradecanoylamino]pentyl}-amide β-Benzyl Ester (26**, Figure 8**).** To a stirred solution of aspartic acid derivative **25** (363 mg, 0.57 mmol) and amine **13** (250 mg, 0.57 mmol) in CH_2Cl_2 (6 mL) at 0 °C were added successively 1-hydroxy-7-azabenzotriazole (HOAt) (94 mg, 0.69 mmol) and *N*,*N*′-diisopropylcarbodiimide (109 μL, 0.69 mmol). The reaction mixture was stirred for 1 h at 0 °C and overnight at room temperature. The mixture was then diluted with CH_2Cl_2 (20 mL) and water (20 mL). The organic phase was separated and washed with 1 N HCl followed by saturated aqueous $NaHCO_3$. The organic phase was dried over $MgSO_4$. The solvent was evaporated and the residue was purified by flash chromatography on silica gel (CH_2Cl_2/acetone 3:1 → 2:1) to give **26** (436 mg; 72%) as a white solid (less than 5% 2b(*S*)-epimer): R_f = 0.27 (CH_2Cl_2/acetone 5:1); mp 106−108 °C; $[\alpha]_D$ −7 (*c* 0.1; $CHCl_3$); ν_{max} (KBr)/cm^{-1} 3288, 1729, 1637, 1552, 1466, 1172; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.25−7.40 (m, 10H, Ph), 6.97 (br d, 1H, *J* = 8.1 Hz, NH), 6.79 (br t, 1H, *J* = 5 Hz, NH), 6.53 (br d, 1H, *J* = 7.5 Hz, NH), 5.07−5.18 (m and AB, 3H, C*H*$_2$Ph, H-3), 4.73 (m, 1H, H-2b), 4.54 (AB, 2H, *J* = 11.3 Hz, C*H*$_2$Ph), 3.86 (m, 2H, H-2a, H-3″), 3.4−3.6 (m, 2H, 2 H-1a), 3.19 (m, 2H, 2 H-5a), 3.01 (dd, 1H, *J* = 4.7, 16.9 Hz, H-3bB), 2.9 (br, 1H, OH), 2.67 (dd, 1H, *J* = 6.0, 16.9 Hz, H-3bA), 2.43 (m, 4H, 2 H-2″, 2 H-2), 2.28 (t, 2H, *J* = 7.5 Hz, 2 H-2′), 1.4−1.7 (m) and 1.2−1.4 (m) (62 H, 31 CH_2), 0.88 (m, 9H, 3 CH_3); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 173.8, 172.3, 171.9, 170.5, 170.3, 138.4, 135.4, 128.7, 128.5, 128.3, 128.0, 76.9, 71.5, 71.3, 66.9, 65.2, 51.5, 49.5, 41.8, 39.4, 35.6, 34.5, 34.1, 32.0, 29.7, 29.4, 29.3, 28.2, 25.7, 25.3, 25.2, 25.1, 22.8, 14.2; MS-IS 1071.0 $[M + Na]^+$, 1049.0 $[M + H]^+$. Anal. ($C_{63}H_{105}N_3O_9$) C, H, N.

***N*-[(*R*)-3-Dodecanoyloxytetradecanoyl]-D-aspartic Acid, α-*N*-{(4*R*)-5-Hydroxy-4-[(*R*)-3-benzyloxytetradecanoylamino]pentyl}-amide (27).** To a solution of compound **26** (2.53 g; 2.4 mmol, less than 5% 2b(*S*)-epimer) in 1:1 (v/v) EtOH/EtOAc (150 mL) was added Et_3N (4 mL) and the mixture was hydrogenated in the presence of 10% Pd−C (120 mg) at room temperature under H_2 (atmospheric pressure) for 2 h. The catalyst was removed by filtration and the filtrate was concentrated in vacuo. The white solid was taken in 1:1 *i*-PrOH/CH_2Cl_2 (100 mL) and the mixture was stirred in the presence of Amberlite IR-120 (H^+) ion-exchange resin (5 mL) for 10 min. The resin was removed by filtration and the filtrate was concentrated in vacuo and dried under vacuum to give homogeneous acid **27** (2.25 g; 97%) as a white solid: mp 115−117 °C; $[\alpha]_D$ +8 (*c* 1.16; $CHCl_3$); ν_{max} (KBr)/cm^{-1} 3292, 3096, 1729, 1702, 1641, 1547; ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 173.9, 172.6, 171.0, 170.5, 138.1, 128.6, 128.1, 77.0, 71.7, 71.4, 64.6, 51.4, 49.7, 41.8, 39.3, 36.5, 34.6, 34.2, 32.0, 29.8, 29.5, 28.0, 25.4, 25.2, 22.8, 14.2; MS-IS 959.0 $[M + H]^+$. Anal. ($C_{56}H_{99}N_3O_9$) C, H, N.

***N*-[(*R*)-3-Dodecanoyloxytetradecanoyl]-D-aspartic Acid, α-*N*-{(4*R*)-5-Hydroxy-4-[(*R*)-3-hydroxytetradecanoylamino]pentyl}-amide (28).** Compound **26** (417 mg, 0.4 mmol, containing ∼25% 2b(*S*)-epimer) was hydrogenated for 3 h in a 1:1 mixture of MeOH and EtOAc (36 mL) in the presence of 10% Pd−C (20 mg) at room temperature under H_2 (atmospheric pressure). The catalyst was then removed by filtration and washed with a 4:1 mixture of CH_2Cl_2/MeOH (50 mL). The filtrate was concentrated, thus providing free acid **28** as a white solid (345 mg, quant.). Compound **28** was purified by HPLC under the same conditions as compound **1(*R/S)**: R_f = 0.30 (CH_2Cl_2/MeOH 9:1 containing 0.5% AcOH); mp 135−137 °C; ν_{max} (KBr)/cm^{-1} 3450, 3301, 2957, 2922, 2852, 1735, 1637, 1458, 1377, 1235, 1176; ^{1}H NMR (250 MHz, $CDCl_3$/CD_3-OD 7:1; major epimer) δ 7.59 (d, 1H, *J* = 8.5 Hz, NH), 7.47 (t, 1H, *J* = 6 Hz, NH), 7.20 (d, 1H, *J* = 8.5 Hz, NH), 5.17 (m, 1H, H-3), 4.68 (m, 1H, H-2b), 3.8−4.0 (2m, 2H, H-3″, H-2a), 3.57 (dd, 1H, *J* = 4.1, 11.3 Hz, H-1aB), 3.47 (dd, 1H, *J* = 5.0, 11.3 Hz, H-1aA), 3.07−3.34 (m, 2H, 2 H-5a), 2.7−2.85 (m, 2H, 2 H-3b), 2.2−2.55 (m, 6H, 3 CH_2), 1.2−1.65 (m, 62 H, 31 CH_2), 0.88 (t, 9H, 3 CH_3); ^{13}C NMR (62.9 MHz, $CDCl_3$/CD_3OD 7:1; major epimer) δ 173.9, 173.8, 173.2, 170.5, 71.1, 68.7, 64.1, 51.1, one signal hidden by CD_3OD signal, 43.3, 41.2, 39.1, 38.9, 37.1, 35.8, 34.3, 34.2, 31.7, 28.9−29.5, 27.5, 25.5, 25.4, 25.0, 24.8, 22.5, 13.9; MS-IS: *m*/*z* 868.7 $[M + H]^+$, 890.7 $[M + Na]^+$, 912.7 $[M - H + 2Na]^+$. Anal. ($C_{49}H_{93}N_3O_9$) C, H, N.

***N*-[(*R*)-3-Dodecanoyloxytetradecanoyl]-D-homoserine, *N*-{(4*R*)-5-Hydroxy-4-[(*R*)-3-benzyloxytetradecanoylamino]pentyl}-amide (29).** To a stirred solution of acid **27** (1.71 g, 1.78 mmol, less than 5% 2b(*S*)-epimer) in THF (12 mL) at 0 °C were added successively *N*-methylmorpholine (196 μL, 1.78 mmol) and isobutyl chloroformate (232 μL, 1.78 mmol). Stirring was continued at room temperature for 30 min and the solution was again cooled to 0 °C. A solution of $NaBH_4$ (135 mg, 3.57 mmol) in H_2O (4.5 mL) was added (**CAUTION**: evolution of gas); the solution was diluted with H_2O (4.5 mL) and THF (5 mL) and stirred at room temperature for 5 min. The organic solvent was then partially evaporated under reduced pressure, CH_2Cl_2 (25 mL) was added, and the residual aqueous phase was neutralized with 1 N HCl. The organic layer was separated and dried over $MgSO_4$. The solvent was evaporated and the residue was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH, 14:1) to give homogeneous diol **29** (1.24 g, 74%) as a white solid: R_f = 0.27 (CH_2Cl_2/MeOH 12:1); mp 112−113 °C; ν_{max} (KBr)/cm^{-1} 3293, 1729, 1643, 1560, 1467, 1378; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.28−7.40 (m, 5H, Ph), 6.91 (br d, 1H, *J* = 7.6 Hz, NH), 6.87 (br, 1H, NH), 6.61 (d, 1H, *J* = 7.6 Hz, NH), 5.16 (quintet, 1H, H-3), 4.45−4.62 (m and AB, 3H, J_{AB} = 11.1 Hz, H-2b and C*H*$_2$Ph), 3.87 (m, 2H, H-2a, H-3″), 3.45−3.75 (m, 4H, 2 CH_2), 3.23 (m, 2H, CH_2), 2.40−2.55 (m, 4H, 2 H-2″, 2 H-2), 2.29 (t, 2H, *J* = 7.6 Hz, 2 H-2′), 1.96 (m, 1H, H-3bB), 1.75 (m, 1H, H-3bA), 1.45−1.70 (m, 10H) and 1.2−1.45 (m, 54H) (32 CH_2), 0.88 (m, 9H, 3 CH_3); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 173.7, 172.2, 171.7, 171.0, 138.3, 128.5, 128.0, 76.9, 71.6, 71.2, 64.8, 58.6, 51.4, 50.9, 41.7, 39.3, 36.0, 34.6, 34.1, 32.0, 29.3−29.8, 28.3, 25.6, 25.3, 25.1, 22.8, 14.2; MS-IS 945.0 $[M + H]^+$. Anal. ($C_{56}H_{101}N_3O_8$) C, H, N.

***O*-Bis(benzyloxy)phosphoryl *N*-[(*R*)-3-Dodecanoyloxytetradecanoyl]-D-homoserine, *N*-{(4*R*)-5-Bis(benzyloxy)phosphoryloxy-4-[(*R*)-3-benzyloxytetradecanoylamino]pentyl}amide (30).** To a stirred solution of **29** (1.02 g, 1.08 mmol, less than 5% 2b(*S*)-epimer) and 1*H*-tetrazole (454 mg, 6.48 mmol) in THF (46 mL) at room temperature was added dibenzyl diethylphosphoramidite (85%, 1.5 mL, 3.78 mmol). Stirring was continued for 30 min at room temperature. The solution was cooled to −20 °C. A solution of *m*CPBA (57−86%, 1.32 g, ∼7.7 mmol) in CH_2Cl_2 (30 mL) was added and the solution was stirred for 45 min at room temperature. Saturated aqueous sodium thiosulfate (25 mL) was then added and the mixture was stirred for 10 min. The mixture was diluted with ether (50 mL) and the organic phase was separated. The organic layer was washed successively with saturated $Na_2S_2O_3$ (5×), saturated $NaHCO_3$ (2 ×), and 1 N HCl (1 ×) and the solution was dried over $MgSO_4$. The solvent was removed in vacuo and the residual product was purified by flash chromatography on silica gel (CH_2Cl_2/acetone, 4:1 → 3:1), which provided homogeneous **30** (1.38 g, 87%) as a colorless oil: $R_f = 0.23$ (CH_2Cl_2/acetone 5:1); $[\alpha]_D$ +7 (*c* 1.25, $CHCl_3$); ν_{max} (KBr)/cm^{-1} 3286, 3065, 1727, 1633, 1555, 1463, 1380, 1266, 1019; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.2−7.4 (m, 25H, Ph), 7.06 (t, 1H, $J = 5$ Hz, NH), 6.76 (d, 1H, $J = 8$ Hz, NH), 6.72 (d, 1H, $J = 8$ Hz, NH) 5.15 (quintet, 1H, H-3), 4.93−5.08 (m, 8H, 4 POCH_2Ph), 4.51 (br q, 1H, H-2b), 4.48 (s, 2H, OCH_2Ph), 3.75−4.1 (m, 6H), 3.15 (m, 2H, 2 H-5a), 2.33−2.47 (m, 4H, 2 H-2″, 2 H-2), 2.25 (t, 2H, $J = 7.5$ Hz, 2 H-2′), 2.04 (m, 2H, 2 H-3b), 1.40−1.65 (m, 10H) and 1.20−1.40 (m, 54H) (32 CH_2), 0.88 (m, 9H, 3 CH_3); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 173.5, 171.3, 170.6, 169.7, 138.6, 135.7, 128.7, 128.4, 128.1, 127.8, 76.6, 71.4, 71.1, 69.6 (m), 68.9 (d), 64.7 (d), 50.0, 48.6 (d), 41.6, 39.2, 34.6, 34.4, 34.3, 33.3 (br), 32.0, 29.7, 29.4, 27.9, 25.3, 25.1, 22.8, 14.2; MS-IS 1483.0 $[M + NH_4]^+$, 1465.0 $[M + H]^+$. Anal. ($C_{84}H_{127}N_3O_{14}P_2$) C, H, N, P.

***O*-Dihydroxyphosphoryl *N*-[(*R*)-3-Dodecanoyloxytetradecanoyl]-D-homoserine, *N*-{(4*R*)-5-Dihydroxyphosphoryloxy-4-[(*R*)-3-hydroxytetradecanoylamino]pentyl}amide (1(*R)).** Compound **30** (1.14 g; 0.78 mmol, less than 5% 2b(*S*)-epimer) in EtOH (70 mL) was hydrogenated for 3 h in the presence of 10% Pd−C (150 mg) at room temperature under hydrogen (atmospheric pressure). The catalyst was removed by filtration and the filtrate was concentrated in vacuo. The residual white solid was dried under vacuum to give compound **1(*R)** as the free acid (764 mg; 97%); a sample was purified by HPLC under the same conditions as compound **1(*R/S*)**: mp 155−157 °C; $[\alpha]_D$ +6 (*c* 1.0, $CHCl_3$); ν_{max} (KBr)/cm^{-1} 3298, 1733, 1646, 1559, 1467, 1378, 1204, 1024; ^{1}H NMR (250 MHz, $CDCl_3$/CD_3OD 4:1) δ 5.11 (m, 1H, H-3), 4.39 (m, 1H, H-2b), 3.77−4.05 (m, 6H), 2.98−3.33 (m, 2H), 2.15−2.49 (m, 6H, 3 CH_2), 1.95 (m, 2H, 2 H-3b), 1.45−1.60 (m, 10H) and 1.1−1.3 (m, 54H) (32 CH_2), 0.80 (m, 9H, 3 CH_3); ^{13}C NMR (62.9 MHz, $CDCl_3$/CD_3OD 4:1) δ 173.7, 172.8, 171.3, 170.6, 70.8, 68.5, 67.3 (d), 62.7 (d), 50.0, 48.8, 42.9, 40.8, 38.7, 36.9, 34.1, 33.9, 32.6 (d), 31.5, 29.0−29.2, 28.8, 27.3, 25.2, 25.0, 24.8, 24.6, 22.3, 13.6; MS-IS 1015.0 $[M + H]^+$. Anal. ($C_{49}H_{97}N_3O_{14}P_2$) C, H, N, P.

***N*-[(*R*)-3-Dodecanoyloxytetradecanoyl]-L-aspartic Acid, α-*N*-{(4*R*)-5-Hydroxy-4-[(*R*)-3-benzyloxytetradecanoylamino]pentyl}-amide β-benzyl Ester (26(*S)).** H-L-Asp(OBn)-OH (537 mg, 2.4 mmol) was *N*-acylated with (*R*)-3-dodecanoyloxytetradecanoic acid **8** (2.4 mmol) by way of its mixed anhydride under the same conditions as described for **25**, thus affording compound **25(*S)** (1.19 g, 78%; white solid; NMR spectra nearly identical to those of **25**). Compound **25(*S)** (654 mg, 1.03 mmol) was coupled with amine **13** (450 mg, 1.03 mmol) in the presence of EDCI and HOAt, under the same conditions as described for the preparation of **26**. The reaction afforded compound **26(*S)** (741 mg, 68%) as a white solid (less than 10% of 2b(*R*)-epimer): mp 95−99 °C; $[\alpha]_D$ −7 (*c* 1.05, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.25−7.45 (m, 10H, Ph), 7.01 (br t, 1H, $J = 5.5$ Hz, NH), 6.54 (br d, 1H, $J = 8$ Hz, NH), 5.22 (m, 1H, H-3), 5.10 (s, 2H, CH_2Ph), 4.78 (m, 1H, H-2b), 4.54 (AB, 2H, $J = 11.0$ Hz, CH_2Ph), 4.05 (m, 1H) and 3.85 (quintet, 1H) (H-2a, H-3″), 3.32−3.58 (m, 3H, 2 H-1a, H-5aB), ∼3.14 (m, 1H, H-5aA), 3.09 (dd, 1H, $J = 4.2$, 17.1 Hz, H-3bB), 2.98 (br t, 1H, OH), 2.66 (dd, 1H, $J = 5.9$, 17.1 Hz, H-3bA), 2.35−2.55 (m, 4H, 2 H-2″, 2 H-2), 2.27 (t, 2H, $J = 7.5$ Hz, 2 H-2′), 1.4−1.7 (m) and 1.2−1.4 (m) (62 H, 31 CH_2), 0.88 (m, 9H, 3 CH_3); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 174, 172.4, 170.4, 170.2, 138.3, 135.4, 128.7, 128.6, 128.5, 128.2, 128.0, 76.9, 71.5, 71.2, 66.9, 65.7, 51.5, 49.2, 42.1, 41.8, 39.7, 35.8, 34.7, 34.5, 34.1, 32.0, 28.8−29.8, 25.4, 25.2, 25.1, 22.8, 14.2.

***O*-Dihydroxyphosphoryl *N*-[(*R*)-3-Dodecanoyloxytetradecanoyl]-L-homoserine, *N*-{(4*R*)-5-Dihydroxyphosphoryloxy-4-[(*R*)-3-hydroxytetradecanoylamino]pentyl}amide (1(*S)).** Compound **26(*S)** was submitted to the same sequence of reactions as its epimer **26**, namely partial hydrogenolysis (→ **27(*S)**, ∼100%), reduction of the carboxylic acid function by way of a mixed anhydride (→ **29(*S)**, 64%), bis-phosphorylation (→ **30(*S)**, 92%), and hydrogenolysis (95%), thus affording diphosphate **1(*S)**. A sample was purified by HPLC under the same conditions as compound **1(*R/S)**: ^{13}C NMR (62.9 MHz, $CDCl_3$/CD_3OD) δ 173.8, 173.1, 171.5, 170.8, 71.1, 68.9, 67.7 (d), 63.0 (d), 50.2, 49.1 (d), 43.2, 41.0, 39.0, 37.1, 34.3, 34.2, 32.9 (d), 31.8, 29.0−29.5, 27.5, 25.4, 25.1, 24.9, 22.5, 13.8; MS-IS 1015.0 $[M + H]^+$.

***N*-[(*R*)-3-Dodecanoyloxytetradecanoyl]-D-aspartic Acid, α-*N*-{(4*R*)-5-(Benzyloxymethoxy)-4-[(*R*)-3-benzyloxytetradecanoylamino]pentyl}amide β-Benzyl Ester (31).** IIDQ (611 mg, 2.01 mmol) was added to a solution of acid **25** (1.06 g, 1.68 mmol) in dry CH_2Cl_2 (81 mL); after stirring for 15 min at room temperature, a solution of amine **15** (1.03 g; 1.84 mmol) in CH_2Cl_2 (35 mL) was added and the reaction mixture was stirred for 3 h. The solution was concentrated and the residue was purified by flash chromatography on silica gel (light petroleum/EtOAc 2:1 → 1:1), which afforded compound **31** (1.30 g; 66%; containing ∼25% 2b(*S*)-epimer) as a white solid: $R_f = 0.40$ (light petroleum/EtOAc 1:1); mp 77−79 °C; ν_{max} (KBr)/cm^{-1} 3289, 2922, 2851, 1729, 1637, 1557, 1173, 731, 695; ^{1}H NMR (250 MHz, $CDCl_3$; major epimer) δ 7.20−7.45 (m, 15H, Ph), 7.03 (d, 1H, $J = 8.3$ Hz, NH), 6.86 (m, 1H, $J = 5.7$ Hz, NH), 6.53 (d, 1H, $J = 8.5$ Hz, NH), 5.05−5.25 (m and AB, 3H, CH_2Ph, H-3), 4.77 (m, 1H, H-2b), 4.45−4.65 (m, 6H, 2 CH_2Ph, OCH_2O), 4.08 (m, 1H, H-2a), 3.84 (m, 1H, H-3″), 3.57 (dd, 1H, $J = 3.7$, 10.0 Hz, H-1aB), 3.43 (dd, 1H, $J = 4.4$, 10.0 Hz, H-1aA), 3.20 (m, 2H, 2 H-5a), 3.02 (dd, 1H, $J = 4.4$, 16.8 Hz, H-3bB), 2.66 (dd, 1H, $J = 6.4$, 16.8 Hz, H-3bA), 2.36−2.49 (m, 4H, 2 H-2″, 2 H-2), 2.27 (t, 2H, $J = 7.5$ Hz, 2 H-2′), 1.45−1.70 (m, 10H) and 1.20−1.40 (m, 54H) (32 CH_2), 0.88 (m, 9H, 3 CH_3); ^{13}C NMR (62.9 MHz, $CDCl_3$; major epimer) δ 173.6, 172.0, 171.2, 170.1, 138.4, 137.7, 135.5, 128.6, 128.5, 128.3, 127.8, 127.7, 94.9, 76.8, 71.4, 71.1, 69.6, 66.8, 49.3, 48.5, 41.8, 39.5, 35.9, 35.7, 34.5, 34.1, 32.0, 29.7, 29.4, 29.2, 25.2, 25.1, 22.7, 14.2; MS-IS 1169.0 $[M + H]^+$. Anal. ($C_{71}H_{114}N_3O_{10}$) C, H, N.

***N*-[(*R*)-3-Dodecanoyloxytetradecanoyl]-D-aspartic Acid, α-*N*-{(4*R*)-5-(Benzyloxymethoxy)-4-[(*R*)-3-benzyloxytetradecanoylamino]pentyl}amide (32).** To a solution of compound **31** from the preceding experiment (1.05 g; 0.90 mmol) in a 1:1 mixture of EtOH and EtOAc (65 mL) was added Et_3N (1.5 mL); the mixture was hydrogenated for 1 h in the presence of 10% Pd−C (50 mg) at room temperature under H_2 (atmospheric pressure). The catalyst was removed by filtration and the filtrate was concentrated in vacuo. The white solid was taken in *i*PrOH/CH_2Cl_2 1:1 (50 mL) and stirred for 10 min in the presence of Amberlite IR-120 (H^+) ion-exchange resin (3 mL). The resin was removed by filtration, the filtrate was concentrated, and the residue dried under vacuum to give acid **32** (956 mg; 99%) as a white solid. $R_f = 0.50$ (CH_2Cl_2/MeOH, 9:1); mp 93−95 °C; ν_{max} (KBr)/cm^{-1} 3291, 1728, 1702, 1641, 1549, 1174, 695; ^{1}H NMR (250 MHz, $CDCl_3$; major epimer) δ 7.20−7.43 (m, 10H, Ph), 7.14 (d, 1H, $J = 8.0$ Hz, NH), 7.04 (br t, 1H, $J = 5.4$ Hz, NH), 6.85 (d, 1H, $J = 8.6$ Hz, NH), 5.16 (m, 1H, H-3), 4.75 (m, 1H, H-2b), 4.45−4.65 (m, 6H, 2 CH_2Ph, OCH_2O), 4.00 (m, 1H, H-2a), 3.84 (m, 1H, H-3″), 3.58 (dd, 1H, $J = 3.7$, 10.0 Hz, H-1aB), 3.43 (dd, 1H, $J = 3.4$, 10.0 Hz, H-1aA), 3.32 (m, 1H, H-5aB), 3.08 (m, 1H, H-5aA), 2.91 (dd, 1H, $J = 4.6$, 16.8 Hz, H-3bB), 2.64 (dd, 1H, $J = 7.6$, 16.8 Hz, H-3bA), 2.35−2.52 (m, 4H, 2 H-2″, 2 H-2), 2.27 (t, 2H, $J = 7.4$ Hz, 2 H-2′), 1.45−

1.65 (m, 10H) and 1.20−1.40 (m, 54H) (32 CH_2), 0.88 (m, 9H, 3 CH_3); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 173.6, 173.4, 171.9, 170.7, 170.2, 138.2, 137.6, 128.5, 128.4, 127.8, 94.9, 76.8, 71.4, 71.2, 69.6, 69.2, 49.6, 49.0, 41.6, 39.3, 36.6, 34.5, 34.0, 32.0, 29.7, 29.4, 29.2, 28.8, 25.7, 25.3, 25.1, 22.7, 14.1; MS-IS 1117.0 $[M + K]^+$, 1079.0 $[M + H]^+$. Anal. ($C_{64}H_{108}N_3O_{10}$) C, H, N.

***N*-[(*R*)-3-Dodecanoyloxytetradecanoyl]-D-homoserine, *N*-{(4*R*)-5-(Benzyloxymethoxy)-4-[(*R*)-3-benzyloxytetradecanoylamino]pentyl}amide (33).** To a stirred solution of acid **32** from the preceding experiment (855 mg, 0.79 mmol) in THF (5 mL) at 0 °C were added successively *N*-methylmorpholine (86 μL, 0.79 mmol) and isobutyl chloroformate (103 μL, 0.79 mmol). Stirring was continued for 30 min at room temperature, and the solution was then cooled to 0 °C. A solution of $NaBH_4$ (60 mg, 1.58 mmol) in H_2O (2 mL) was added (**CAUTION**: evolution of gas); the reaction mixture was further diluted with H_2O (2 mL) and THF (2.5 mL) and stirred for 5 min at room temperature. The organic solvent was then partially evaporated; CH_2Cl_2 (25 mL) was added and the aqueous phase was neutralized with 1 N HCl. The organic layer was separated and dried over $MgSO_4$. The solvent was evaporated and the residue was purified by flash chromatography on silica gel (CH_2Cl_2/acetone, 4:1) to give **33** (387 mg, 46%) as a white solid. Further elution (CH_2Cl_2/MeOH 9:1) then gave recovered starting material **32** (193 mg, 23%). Compound **33**: $R_f = 0.35$ (CH_2Cl_2/acetone 4:1); mp 90−92 °C; ν_{max} (KBr)/cm^{-1} 3474, 3296, 1728, 1640, 1613, 1054, 695; ^{1}H NMR (250 MHz, $CDCl_3$, major epimer) δ 7.42 (m, 1H, NH), 7.18−7.36 (m, 10H, Ph), 7.12 (m, 1H, NH), 6.75 (d, 1H, $J = 8.3$ Hz, NH), 5.19 (m, 1H, H-3), 4.45−4.65 (m, 7H, 2 CH_2Ph, OCH_2O, H-2b), 4.25 (m, 1H, OH), 4.05 (m, 1H, H-2a), 3.84 (m, 1H, H-3″), 3.58 (m, 3H, 2 H-4b, H-1aB), 3.44 (dd, 1H, $J = 4.2$, 9.8 Hz, H-1aA), 3.23 (m, 2H, 2 H-5a), 2.35−2.52 (m, 4H, 2 H-2″, 2 H-2), 2.27 (t, 2H, $J = 7.3$ Hz, 2 H-2′), 1.98 (m, 1H, H-3bB), 1.70 (m, 1H, H-3bA), 1.45−1.65 (m, 10H) and 1.10−1.40 (m, 54H) (32 CH_2), 0.88 (m, 9H, 3 CH_3); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 173.3, 171.5, 171.3, 170.7, 138.3, 137.6, 128.4, 128.3, 127.7, 127.6, 94.8, 76.6, 71.3, 70.9, 69.4, 58.3, 50.5, 48.6, 41.6, 41.4, 39.2, 36.1, 34.4, 34.2, 34.0, 31.9, 29.6, 29.3, 29.1, 25.6, 25.1, 25.0, 22.6, 14.1; MS-IS 1103.0 $[M + K]^+$, 1065.0 $[M + H]^+$. Anal. ($C_{64}H_{110}N_3O_9$) C, H, N.

***O*-Bis(benzyloxy)phosphoryl *N*-[(*R*)-3-Dodecanoyloxytetradecanoyl]-D-homoserine, *N*-{(4*R*)-5-(Benzyloxymethoxy)-4-[(*R*)-3-benzyloxytetradecanoylamino]pentyl}amide (34).** To a stirred solution of compound **33** from the preceding experiment (313 mg, 0.29 mmol) and 1*H*-tetrazole (62 mg, 0.88 mmol) in THF (12 mL) at room temperature was added dibenzyl diethylphosphoramidite (85%, 267 μL, 0.67 mmol). Stirring was continued for 30 min at room temperature and the solution was then cooled to −20 °C. A solution of *m*CPBA (57−86%, 187 mg, ∼1.1 mmol) in CH_2Cl_2 (8 mL) was added and the mixture was stirred for 45 min at room temperature. Saturated aqueous sodium thiosulfate (5 mL) was added, the mixture was stirred for 10 min and then diluted with ether (25 mL), and the organic phase was separated. The organic layer was washed successively with saturated $Na_2S_2O_3$ (5×), saturated $NaHCO_3$ (2×), and 1 N HCl solution (1×); the solution was dried over $MgSO_4$ and the solvent removed in vacuo. Flash chromatography of the residual product on silica gel (CH_2Cl_2/acetone, 4:1 → 3:1) provided **34** (361 mg, 93%) as a white solid: $R_f = 0.46$ (CH_2Cl_2/acetone 4:1); mp 68−70 °C; ν_{max} (KBr)/cm^{-1} 3286, 1727, 1635, 1557, 1455, 1381, 1266, 1204, 1174, 1025, 733, 695; ^{1}H NMR (250 MHz, $CDCl_3$; major epimer) δ 7.20−7.42 (m, 20H, Ph), 7.12 (m, 1H, NH), 6.67 (d, 1H, $J = 7.2$ Hz, NH), 6.61 (d, 1H, $J = 9.1$ Hz, NH), 5.16 (m, 1H, H-3), 4.95−5.12 (m, 4H, 2 POCH_2Ph), 4.45−4.65 (m, 7H, 2 CH_2Ph, OCH_2O, H-2b), 3.95−4.15 (m, 3H, 2 H-4b, H-2a), 3.84 (quintet, 1H, H-3″), 3.57 (dd, 1H, $J = 3.8$, 9.8 Hz, H-1aB), 3.44 (dd, 1H, $J = 4.3$, 9.8 Hz, H-1aA), 3.20 (m, 2H, 2 H-5a), 2.35−2.46 (m, 4H, 2 H-2″, 2 H-2), 2.26 (t, 2H, $J = 7.9$ Hz, 2 H-2′), 2.03 (m, 2H, 2 H-3b), 1.45−1.65 (m, 10H) and 1.15−1.40 (m, 54H) (32 CH_2), 0.88 (m, 9H, 3 CH_3); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 173.5, 171.2, 170.5, 169.7, 138.6, 137.8, 135.7, 128.7, 128.5, 128.1, 127.8, 127.7, 95.0, 76.9, 71.5, 71.1, 69.6, 64.8, 53.5, 50.1, 48.7, 41.8, 39.5, 34.5, 34.2, 33.6, 32.0, 29.7, 29.4, 25.8, 25.3, 25.1, 22.8, 14.2; MS-IS 1325.0 $[M + H]^+$. Anal. ($C_{78}H_{122}N_3O_{12}P$) C, H, N, P.

***O*-Dihydroxyphosphoryl *N*-[(*R*)-3-Dodecanoyloxytetradecanoyl]-D-homoserine, *N*-{(4*R*)-5-Hydroxy-4-[(*R*)-3-hydroxytetradecanoylamino]pentyl}amide (2(**R*)).** Compound **34** from the preceding experiment (288 mg; 0.22 mmol) in a 1:1 EtOH/EtOAc mixture (30 mL) was hydrogenated for 7 h in the presence of 10% Pd−C (60 mg) at room temperature under H_2 (atmospheric pressure). The catalyst was then removed by filtration and the filtrate was concentrated in vacuo. The white solid was dried under vacuum to give homogeneous compound **2** (containing ∼25% 2b(*S*)-epimer) (200 mg; 98%): $R_f = 0.60$ (CH_2Cl_2/MeOH/H_2O 6:4:0.6); mp 150−160 °C (dec); ν_{max} (KBr)/cm^{-1} 3396, 3297, 1728, 1647, 1559, 1467, 1246, 1048, 721; ^{1}H NMR (250 MHz, $CDCl_3$/CD_3OD 4:1, major epimer) δ 5.11 (m, 1H, H-3), 4.30−4.50 (m, 1H, H-2b), 3.70−4.05 (m, 4H), 3.35−3.55 (m, 2H), 3.00−3.25 (m, 2H), 2.15−2.45 (m, 6H, 3 CH_2), 1.95 (m, 2H, 2 H-3b), 1.45−1.60 (m, 10H) and 1.10−1.40 (m, 54H) (32 CH_2), 0.80 (m, 9H, 3 CH_3); ^{13}C NMR (62.9 MHz, $CDCl_3$/CD_3OD 4:1) δ 173.7, 173.1, 171.3, 170.6, 70.9, 68.5, 63.7, 62.6, 50.8, 50.0, 43.0, 40.9, 38.9, 36.9, 34.1, 34.0, 32.7 (br), 31.6, 29.3, 29.2, 29.0, 28.8, 27.7 (br), 25.2, 24.8, 24.6, 22.3, 13.6; MS-IS 957.0 $[M + Na]^+$, 935.0 $[M + H]^+$. Anal. ($C_{49}H_{96}N_3O_{11}P + H_2O$) C, H, N, P.

(3*R*,*S*)-3-[(*R*)-3-Dodecanoyloxytetradecanoylamino]-4-hydroxybutanoic Acid, *N*-{(4*R*)-5-Hydroxy-4-[(*R*)-3-benzyloxytetradecanoylamino]pentyl}amide (35). To a stirred solution of $NaBH_4$ (7.5 mg, 0.20 mmol) in a 4:1 mixture of MeOH and H_2O (0.2 mL) at −15 °C was added slowly compound **26** (52 mg, 49 μmol) in a 1:1 mixture of MeOH and THF (0.4 mL). Stirring was continued at −15 °C for 30 min and at room temperature for 30 min. The solvent was partially evaporated under reduced pressure, the residue was taken up in EtOAc (5 mL), and the residual aqueous phase was neutralized with 1 N HCl. The organic layer was separated, washed with water, and dried over $MgSO_4$. The solvent was evaporated and the residue was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH, 12:1) to give diol **35** (20 mg, 43%, containing ∼30% 3b(*S*)-epimer) as a white solid: $R_f = 0.30$ (CH_2Cl_2/MeOH 12:1); ^{1}H NMR (250 MHz, $CDCl_3$, signals of major epimer) δ 7.28−7.37 (m, 5H, Ph), 7.11 (br m, 1H, NH), 7.01 (br d, 1H, $J = 7.5$ Hz, NH), 6.69 (br d, 1H, NH), 5.16 (m, 1H, H-3), 4.55 (AB, 2H, $J = 11.2$ Hz, CH_2Ph), 4.13 (m, 1H, H-3b), 3.80−3.95 (m, 2H, H-3″, H-2a), 3.71 (dd, 1H, $J = 3.7$, 11.5 Hz, H-4bB), 3.42−3.63 (m, 3H, H-4bA, 2 H-1a), 3.22 (m, 3H, OH, 2 H-5a), 2.36−2.54 (m, 6H, 2 H-2″, 2 H-2, 2 H-2b), 2.30 (t, 2H, $J = 7.3$ Hz, 2 H-2′), 1.45−1.70 (m, 10H) and 1.15−1.40 (m, 54H) (32 CH_2), 0.88 (m, 9H, 3 CH_3); ^{13}C NMR (62.9 MHz, $CDCl_3$, major epimer) δ 173.9, 172.2, 171.9, 170.2, 138.1, 128.5, 127.9, 76.7, 71.4, 71.2, 64.6, 63.9, 51.3, 48.7, 41.9, 41.5, 39.3, 38.0, 34.5, 33.8, 31.9, 29.6, 29.5, 29.3, 29.1, 28.3, 25.2, 25.1, 25.0, 22.6, 14.1; MS-IS 967.0 $[M + Na]^+$, 945.0 $[M + H]^+$.

(3*R*,*S*)-4-Bis(benzyloxy)phosphoryloxy-3-[(*R*)-3-dodecanoyloxytetradecanoylamino]butanoic Acid *N*-{(4*R*)-5-Dibenzyloxyphosphoryloxy-4-[(*R*)-3-benzyloxytetradecanoylamino]pentyl}amide (36). To a stirred solution of diol **35** (20 mg, 21 μmol) and 1*H*-tetrazole (9 mg, 127 μmol) in THF (1 mL) at room temperature was added dibenzyl diethylphosphoramidite (85%, 25 μL, ∼97 μmol). Stirring was continued for 30 min at room temperature and the solution was then cooled to −20 °C. A solution of *m*CPBA (57−86%, 27 mg, ∼160 μmol) in CH_2Cl_2 (0.6 mL) was added and the solution was further stirred for 45 min at room temperature. Saturated aqueous sodium thiosulfate (2 mL) was added, the mixture was stirred for 10 min and then diluted with ether (10 mL), and the organic phase separated. The organic layer was washed successively with saturated $Na_2S_2O_3$ (5×), saturated $NaHCO_3$ (2×), 1 N HCl (1×); the organic solution was dried over $MgSO_4$ and the solvent removed in vacuo. Purification of the residual product by flash chromatography on silica gel (CH_2Cl_2/acetone, 3:1 → 2:1) provided **36** (18 mg, 58%) as a white solid: $R_f = 0.40$ (CH_2Cl_2/acetone 3:1); ^{13}C NMR (62.9 MHz, $CDCl_3$, major epimer) δ 173.5, 171.3, 170.5, 169.7, 138.5, 135.7, 128.8, 128.5, 128.1, 127.9, 76.6, 71.3, 71.0, 69.7, 68.9, 67.3, 48.7, 46.8, 41.6, 39.1, 35.9, 34.6, 34.4,

34.1, 32.0, 29.3−29.8, 28.2, 25.5, 25.3, 25.2, 22.8, 14.2; MS-IS 1482.0 $[M + NH_4]^+$, 1465.0 $[M + H]^+$.

(3*R*,*S*)-4-Dihydroxyphosphoryloxy-3-[(*R*)-3-dodecanoyloxytetradecanoylamino]butanoic Acid, *N*-{(4*R*)-5-Dihydroxyphosphoryloxy-4-[(*R*)-3-hydroxytetradecanoylamino]pentyl}amide (37). Compound **36** (16 mg; 11 μmol) was hydrogenated for 4 h in EtOH (1.5 mL) in the presence of 10% Pd−C at room temperature under H_2 (atmospheric pressure). The catalyst was then removed by filtration and washed with CH_2Cl_2/MeOH 4:1, and the filtrate was concentrated in vacuo. The white solid was dried under vacuum to give homogeneous **37** (10 mg; 90%); a sample was purified by HPLC under the same conditions as described for compound **1(*R*/*S*)**: ^{1}H NMR (250 MHz, $CDCl_3/CD_3OD$ 4:1) δ 5.12 (m, 1H, H-3), 4.30 (m, 1H, H-3b), 3.7−4.04 (m, 6H), 3.15 (m, 2H, 2 H-5a), 2.38 (m, 4H, 2 CH_2), 2.22 (m, 4H, CH_2), 1.35−1.60 (m, 10H) and 1.10−1.35 (m, 54H) (32 CH_2), 0.88 (m, 9H, 3 CH_3); ^{13}C NMR (62.9 MHz, $CDCl_3/CD_3OD$ 4:1, major epimer) δ 173.5, 171.3, 170.5, 169.7, 70.9, 68.6, 67.4, 65.9, 48.7, 46.6, 43.0, 40.9, 38.6, 37.0, 36.0, 34.1, 33.9, 31.6, 29.6, 29.3, 29.0, 28.8, 27.4, 25.2, 24.8, 24.7, 22.3, 13.6; MS-IS 1036.5 $[M + Na]^+$, 1014.5 $[M + H]^+$.

Biological Assays. Experimental Assay of Nitric Oxide Production by Murine Macrophages. Six-week-old male C57/BL6 mice (SPF quality, Charles Rivier, FR) were killed by CO_2 inhalation. The hip, femur, and tibia from the posterior appendage were removed. The bone marrow was extracted from the lumen by injecting Dulbecco's modified Eagle medium (DH) through the bone after cutting both end portions. After washing, the stem cells were resuspended (40 000 cells/mL) in DH medium supplemented with 20% horse serum and 30% L929 cell supernatant. The cell suspension was incubated for 8 d in an incubator at 37 °C under 8% CO_2 and moisture-saturated atmosphere. Macrophages were then detached with ice-cold PBS, washed, and resuspended in DH medium supplemented with 5% fetal calf serum (FCS), amino acids, and antibiotics (DHE medium). The cell density was adjusted to 700 000 cells/mL. Aqueous solutions of the products were serially diluted in DHE medium directly in microtiter plates. The products were tested in triplicate and each microtiter plate comprised a negative control composed of medium. The final volume in each well was 100 μL, and 100 μL of the cell suspension was added to the diluted products, and the cells were incubated for 22 h in an incubator at 37 °C, under 8% CO_2 and a moisture-saturated atmosphere. At the end of the incubation period, 100 μL of supernatant was transferred to another microtiter plate, and the nitrite concentration produced in each supernatant was determined by running a Griess reaction. Griess reagent (100 μL, 5 mg/mL of sulfanilamide + 0.5 mg/mL of *N*-(1-naphthyl)ethylenediamine hydrochloride) in 2.5% aqueous phosphoric acid was added to each well. The microtiter plates were read with a spectrophotometer (SpectraMax Plus, Molecular Devices) at 562 nm against a reference at 690 nm. The nitrite concentration is proportional to the nitric oxide content being formed. The nitrite content is determined on the basis of a standard curve. The results are given as mean value ± standard deviation and plotted as a dose−response curve.

Preparation of Human PBMC and Cell Culture. Peripheral blood from healthy donors (Centre de transfusion, Hôpital Universitaire, Geneva) was centrifuged to get the buffy coat. The buffy coat was mixed with Hanks' balanced saline solution (HBSS, Sigma, Buchs, Switzerland), layered over Ficoll Paque Plus (Amersham Pharmacia) to 1.077 g/mL, and centrifuged (2800 rpm, 20 °C, 25 min). Cells harvested from the interphase were washed twice in HBSS at 800 rpm for 15 min at room temperature, and the pelleted cells were resuspended in HBSS. The cell counts were performed using a Neubauer cell. All cell cultures were performed in RPMI-1640 medium supplemented with penicillin (100 U/mL), streptomycin (100 μg/mL), L-glutamine (2 mmol/L), and 10% fetal calf serum (FCS), all obtained from Sigma. For in vitro stimulation, the cells were cultured at a concentration of 1×10^6 viable cells/well.

Stimulation and Measurement of IL-6 in Culture Supernatants. PBMC were incubated at 37 °C and under 5% CO_2 atmosphere with the following products at the final concentration of 20 μg/mL: medium, RPMI alone; positive control, LPS of *E. coli* (serotype 055:B5, Sigma, St. Louis, MO); product **1**, OM-294-DP; product **2**, OM-294-MP.

The surpernatants of the cultures were harvested after 24 h, and the concentration of IL-6 was measured by an enzyme-linked immunosorbent assay (ELISA) (Human IL-6 ELISA Set, BD OptEIA, San Diego, CA), according to the manufacturer instructions. The detection limit was 10 pg/mL.

Inhibition and Measurement of IL-6 in Culture Supernatants. The PBMC obtained as described above were incubated at 37 °C and under 5% of CO_2 atmosphere for 90 min with product **1** (OM-294-DP) or product **2** (OM-294-MP) at the final concentrations of 0.02, 0.2, 2, and 20 μg/mL then LPS of *E. coli* (serotype 055:B5, Sigma, St. Louis, MO) was added at the concentration of 0.01 μg/mL.

After 24 h of incubation, the plates were centrifuged at 1500 rpm for 8 min, and the supernatant was collected and stored at −20 °C. The concentration of IL-6 was measured by an enzyme-linked immunosorbent assay (ELISA) (Human IL-6 ELISA Set, BD OptEIA, San Diego, CA), according to the manufacturer instructions. The detection limit was 10 pg/mL.

Supporting Information Available: Elemental analysis results, general experimental procedures, and procedures for LAL assays and for the determination of pyrogenicity in the rabbit. This material is available free of charge via the Internet at http://pubs.acs.org.

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