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Synthesis of Phosphoramide Analogues of Sphinganine-1-phosphate

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Abstract: Phosphoramide derivatives **13** and **15** were prepared as analogues of sphinganine-1-phosphate (**4**). Key steps of the synthesis are the alkylation of N-methoxy-N-methlycarboxyamide of the diaminopropanoic acid **7** and the subsequent reduction of the intermediately formed ketone **8** affording the diastereomeric alcohols **9**. Deprotection and phosphorylation of **9** lead to the desired products **13** and **15**.

Sphingosine **1** and sphinganine **2** (fig. 1) are the long-chain bases most abundant in cellular sphingolipids, e. g. ceramide, sphingomyelin, cerebrosides and gangliosides.[1,2] Sphinganine **2** itself is an intermediate in the biosynthesis of sphingolipids whereas sphingosine **1** is generated as a catabolic intermediate in the degradation of ceramide.[3] Studies on the biological function of sphingosine and related structures showed that these molecules are reversible inhibitors of protein kinase C.[4] They may act as endogenous modulators of cell function and possibly as second messengers.[5] Recently, it was demonstrated that low concentrations of sphingosine increase proliferation of quiescent Swiss 3T3 fibroblasts acting in a fundamentally different, protein kinase C-independent pathway.[6]

Sphingosine-1-phosphate **3** and sphinganine-1-phosphate **4** are the initial intermediates in the catabolism of the long-chain sphingoid bases. They are formed by the cytosolic enzyme sphingosine kinase [7,8] which

catalyses the ATP dependent phosphorylation step at the 1-OH-position (fig. 1). The 1-phosphates **3** and **4** are cleaved by the action of a pyridoxal-phosphate dependent lyase to yield a fatty aldehyde and ethanolamine phosphate.[9] Another primary metabolic product of sphinganine-1-phosphate in cultured skin fibroblasts appeared to be sphinganine, indicating the action of a phosphatase.[10] Comparison of the rates of cleavage by the action of the sphingosine-1-phosphate lyase with those of dephosphorylation indicates that dephosphorylation is at least as active as cleavage. The fast turnover of sphinganine-1-phosphate certainly favours a second-messenger role of this lipid intermediate.

The phosphorylated sphingoid bases are not only intermediary catabolites but also bioactive lipids with important functions including elevation of phosphatidic acid levels [11] and activation of the DNA binding activity of AP-1.[12] Sphingosine-1-phosphate strongly mimics PDGF (platelet-derived growth factor)- receptor induced chemotactic signal transduction favoring actin filament disassembly. This excessive and prolonged signaling results in a marked inhibition of cell spreading, of extension of the leading lamellae toward PDGF, and of chemotaxis toward PDGF.[13]

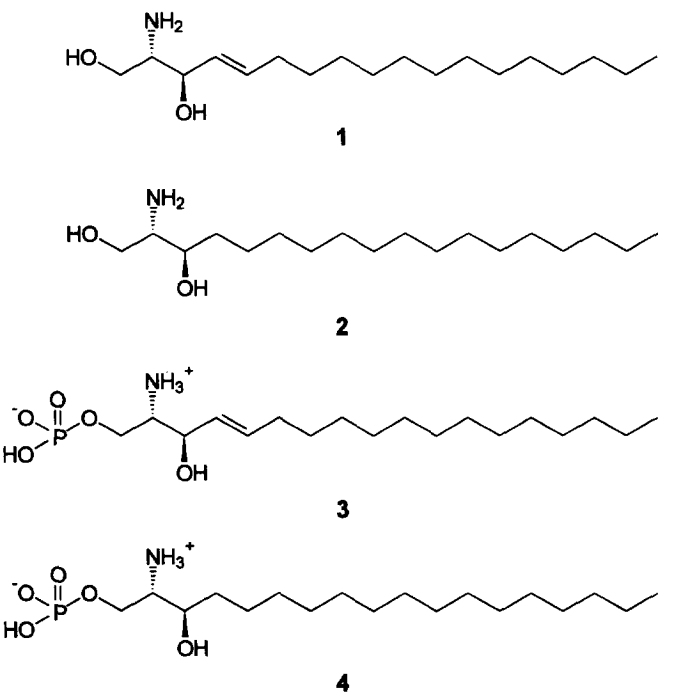
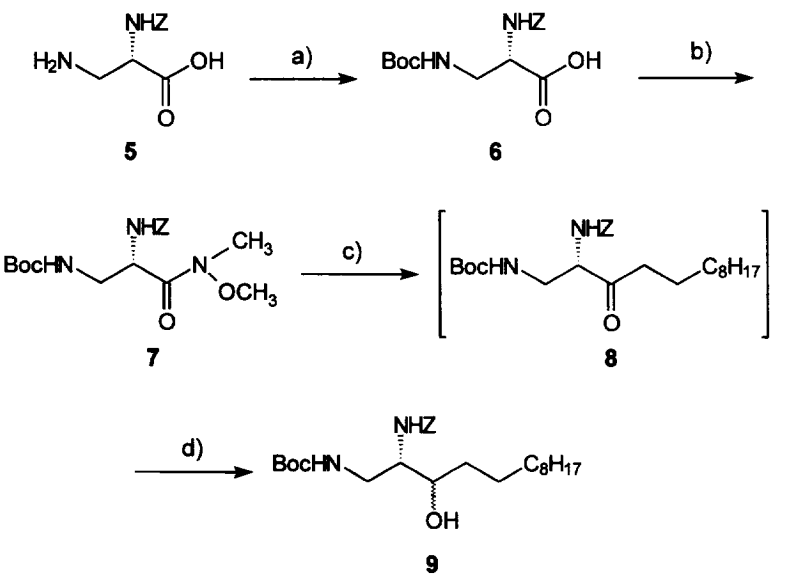


Figure 1

Furthermore, mitogenic concentrations of sphingosine-1-phosphate stimulate production of inositol phosphates, which can be inhibited by pertussis toxin, while the response to bradykinin is not effected. Sphingosine-1-phosphate decreases cellular cAMP levels and also causes a drastic decrease in isoproterenol- and forskolin-stimulated cAMP accumulation. These results suggest that some of the sphingosine-1-phosphate-induced signaling pathways are mediated by G proteins that are substrates for pertussis toxin.[14]

Sphingosine-1-phosphate induces a transient increase in intracellular free calcium independent of extracellular calcium concentrations.[15,16] It was demonstrated that sphingosine-1-phosphate inhibits motility of melanoma cells in very low concentrations (10-100 nM), in which sphingosine shows no inhibitory effect.[17,18] These tools suggest that sphingosine-1-phosphate may have antimetastatic and inflammatory properties.
Therefore, these findings propose that sphingosine-1-phosphate (**3**) and sphinganine-1-phosphate (**4**) have appropriate properities to function as intracellular lipid second messengers that are involved in calcium release and the regulation of the cell growth induced by sphingosine (**1**).

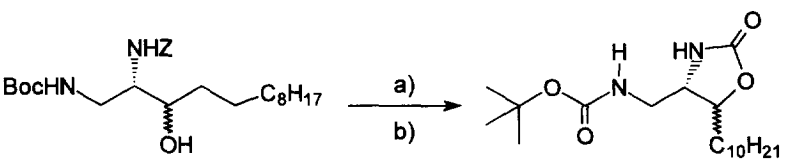
In this paper we describe the synthesis of **13** and **15** as structural analogues of sphinganine-1-phosphate (**4**) modified in the headgroup whereas sphingosine-1-phosphate (**3**) and sphinganine-1-phosphate (**4**) are subjected to fast turnover. **13** and **15** are metabolically resistant. Therefore, they may be suitable tools to study the intracellular functions of sphingosine-1-phosphate (**3**) and sphingosine-1-phosphate (**4**). Compared to natural sphinganine-1-phosphate (**4**), the P-O bond of the phosphoric acid ester in the 1-position of the sphingoid backbone is substituted by a P-N bond. Phosphorylated amines are close analogues of the isosteric phosphate esters with respect to geometry, bond lengths and hydrogen bonding capacity.[19] Therefore, a number of phosphorylated amino analogues of metabolic intermediates have been synthesized.[20] The major drawback of the phosphoramides and phosphoramidates consists in their acid lability. Their resistance towards enzymes transferring phosphoryl groups is varying and depends on the enzyme.[19]
In order to clarify and investigate the biological functions of the metabolically labil sphingosine-1-phosphate and sphinganine-1-phosphate and in addition to the synthesis of the corresponding phosphonates analogues [21], we synthesized the phosphoramides **13** and **15**.



Scheme 1: a) Boc_2O, dioxane/water, 80%; b) EtOCOCl, THF, N, O-dimethyl hydroxylamine hydrochloride, THF, 62%; c) decylmagnesium bromide, THF; d) sodium borohydride, 2-propanol, 48%.

The synthesis started with protection of the N_α-benzyloxycarbonyl-L-2,3-diaminopropanoic acid (**5**) with tert.-butyl dicarbonate and triethylamine in dioxane/water following previous reports [22] (scheme 1).
Treatment of the N_α-benzyloxycarbonyl-N_β-tert.-butoxycarbonyl-L-2,3-diaminopropanoic acid (**6**) with N,O-dimethylhydroxylamine hydrochloride in the presence of ethyl chloroformiate and triethylamine according to König and Geiger [23] led to the amide **7**. Alkylation with decylmagnesium bromide following the procedure previously reported [24,25] yielded ketone **8** that was reduced without further purification by sodium borohydride [26,27,28] forming the diastereomeric amino alcohols **9** as 3 : 1 mixture.

The relative configuration of the alcohols **9** was established after transformation into the corresponding oxazolidinones **10** [29,30] (scheme 2). Generally, the coupling constants of cis-isomers (*erythro*) of such 4,5-disubstituted oxazolidinones are greater than those of the trans-isomer (*threo*).[31] According to ^{1}H NMR investigations of **10**, the major diastereomer is *erythro*-configurated.

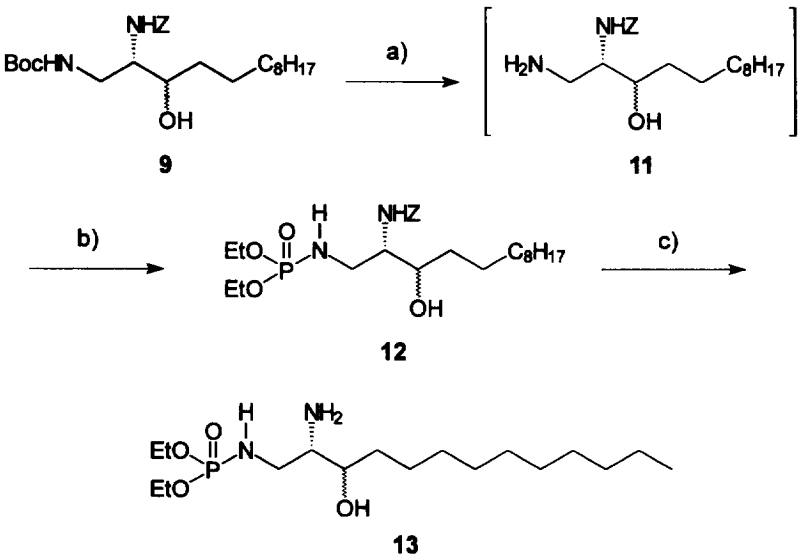


9 **10**

Scheme 2: a) H_2, Pd/C, MeOH, 95%, b) $(Cl_3CO)_2CO$, dichloromethane, triethylamine, 67%

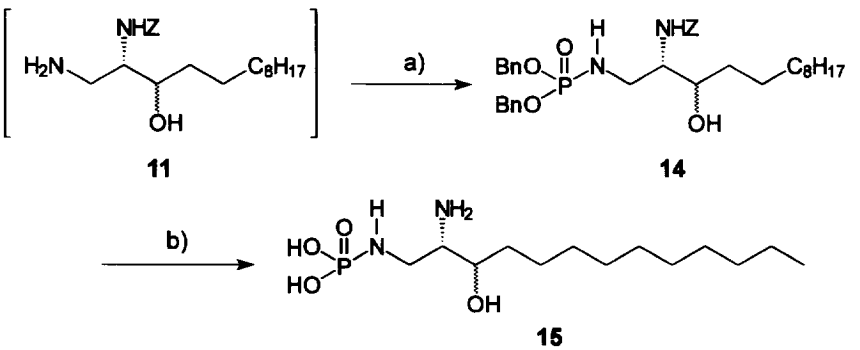
The enantiomeric purity of the alcohols **9** was assured by the *Mosher* method.[32] The ^{1}H NMR spectrum of the corresponding Mosher derivatives of **9** is consistent with a racemization degree minor than 10%.

Removal of the Boc group with HCl gas in methanol and subsequent treatment with saturated sodium hydrogencarbonate solution afforded the crude amines **11** (scheme 3). The amines **11** were converted without further purification into the corresponding phosphoramides **12** by treatment with diethylchloro phosphite in chloroform [33,34] (scheme 3).
Removal of the Z group of compounds **12** was achieved by hydrogenation with palladium on charcoal and afforded compound **13** in good overall yield. All attempts to hydrolyze the phosphoramide diethylesters of **13** with HCl led to the decomposition of the diethylester **13**.



Scheme 3: a) MeOH/HCl, sodium hydrogencarbonate; b) diethyl chlorophosphite, chloroform, 62%; c) H_2, Pd/C, MeOH, 95%.

For the synthesis of the phosphoramide derivatives **15** (scheme 4) the crude amines **11** were treated with dibenzyl phosphite in tetrachloromethane.[35,36]



Scheme 4: a) dibenzyl phosphite, tetrachloromethane, 63%; b) H_2, Pd/C, MeOH, 95%

Deprotection of the dibenzylphosphoryl groups [37] and the benzyloxycarbonyl amino group with hydrogen and palladium on charcoal afforded the target compounds **15** as the phosphoramides.
Biochemical properties of the synthesized compounds will be reported elsewhere.[38]

EXPERIMENTAL

Solvents were purified in the usual way. Water sensitive reactions were carried out in flame dried glassware under argon. Thin layer chromatography: Merck precoated tlc plates, silica gel 60; column chromatography: Kieselgel 60 (Merck, 40-63 µm). Optical rotations. Perkin-Elmer polarimeter P 241. ^{1}H NMR: Bruker AM-250, Bruker AM-400, ^{13}C NMR: Bruker AM-250, ^{31}P NMR: Bruker AMX-300. Mass spectometry: A. E. I. MS-30 and MS-50, ion source 180°C, FAB: Kratos Concept 1H, matrix = m-nitrobenzoic acid. Elemental analyses were performed at the Institute of Organic Chemistry and Biochemistry, Bonn, Microanalytical Department.

2-N-benzyloxycarbonyl-3-N-tert.-butoxycarbonyl-2 (S),3-diaminopropanoic acid (6)

The 2-N-Benzyloxycarbonyl-2 (S),3-diaminopropanoic acid **(5)** (5.0 g, 20.98 mmol) was suspended in dioxane/water (150 ml, 1 : 1 v/v) and triethylamine (4.19 ml, 31.48 mmol) was added. The resulting white suspension was cooled to 0°C and a solution of di-tert.-butyl dicarbonate (5.4 g, 2.98 mmol) in dioxane (50 ml) was added dropwise over 30 min. The mixture was allowed to warm to r. t. and stirred for 6 h at which time TLC analysis showed the reaction to be complete. The solvent was evaporated in vacuo to half its original volume, cooled in an ice-water bath, acidified to pH 5-6 by the slow addition of 0.1 N citric acid (50 ml), and then extracted with dichloromethane (3 x 100 ml). The combined organic extracts were dried over $MgSO_4$, filtered and evaporated under reduced pressure to afford crude **6** which was crystallized from ethyl acetate/ether/petroleum ether to give pure **6**, yield: 6.03 g (85%), m. p. 144-146°C, R_f (n-butanol/AcOH/pyridine/water = 4 : 1 : 1 : 2) = 0.76).

^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.40 (s, br, 9 H, C(C*H*$_3$)$_3$); 3.54 (m, 2 H, C*H*$_2$-CH); 4.36 (m, 1 H, C*H*-CH$_2$); 5.12 (s, 2 H, C*H*$_2$-C$_6$H$_5$); 5.75 (m, 1 H, N*H*Boc); 6.19 (d, br, J = 7.8 Hz, 1 H, N*H*Z); 6.43 (s, br, 1 H, COO*H*); 7.34 (m, 5 H, C$_6$*H*$_5$).

^{13}C NMR (62.89 MHz, $CDCl_3$): δ = 28.48, 28.68, 28.89 (C(*C*H$_3$)$_3$); 46.27 (*C*H$_2$-NH); 55.49 (*C*(CH$_3$)$_3$); 67.64 (*C*H-COOH); 80.67 (O-*C*H$_2$-C$_6$H$_5$); 128.6, 129.10 (*C*$_6$H$_5$); 130.71, 136.18 (*C*$_6$H$_5$); 157.57 (NH-*C*OO-C(CH$_3$)$_3$); 159.92 (NH-*C*OO-CH$_2$-C$_6$H$_5$); 173.98 (*C*OOH).

Analysis: $C_{16}H_{22}N_2O_6$ (338.147) calcd. (%): C 56.78, H 6.56, N 8.30; found (%): C 56.67, H 6.47, N 8.30; MS (FAB-MS): $C_{16}H_{23}N_2O_6$ $[M+H]^+$, calcd.: m/z = 339.155, found: m/z = 339.10.

2-N-benzyloxycarbonyl-3-N-tert.-butoxycarbonyl-2 (S),3-diaminopropanoic acid-N-methoxy-N-methyl-carboxamide (7)

The protected diaminopropanoic acid **6** (3.59 g, 10.56 mmol) and triethylamine (2.8 ml, 21.25 mmol) were dissolved under argon atmosphere in THF (100 ml) and cooled to -23°C. Ethyl chloroformiate (1.1 ml, 11.79 mmol) was added dropwise and the solution was stirred for 15 min at -23°C followed by addition of N, O-dimethylhydroxylamine hydrochloride (2.1 g, 21.25 mmol) and triethylamine (2.8 ml, 21.25 mmol). After 4 h the reaction was complete according to tlc. The precipitate was filtered off and washed with cold THF. The combined filtrates were dried over $MgSO_4$, filtered and evaporated in vacuo affording a yellow oil which was

chromatographed on silica gel (pretrolether/ethyl acetate 2 : 1, R_f = 0.32), yielded 2.47 g (61%) as a colourless oil.

^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.39 (s, br, 9 H, C(CH_3)$_3$); 3.19 (s, 3 H, N-CH_3); 3.32-3.59 (m, br, 2 H, CH_2-CH); 3.76 (s, 3 H, O-CH_3); 4.76 (m, 1 H, CH-CH_2); 4.86 (m, 1 H, N*H*Boc); 5.08 (s, 2 H, CH_2-C_6H_5); 5.85 (d, br, J = 8.2 Hz, 1 H, N*H*Z); 7.33 (m, 5 H, C_6H_5).

^{13}C NMR (62.89 MHz, $CDCl_3$): δ = 21.68, 26.75, 28.94 (C(CH_3)$_3$); 33.09 (N-CH_3); 42.67 (CH_2-CH); 52.44 (C(CH_3)$_3$); 58.70 (HNCH); 67.60 (O-CH_3); 80.27 (O-CH_2-C_6H_5); 128.71, 129.15 (C_6H_5), 131.72, 136.88 (C_6H_5); 156.65 (COO-C(CH_3)$_3$); 171.02 (NH-CO); 171.81 (COO-CH_2-C_6H_5).

Analysis: $C_{18}H_{27}N_3O_6$ x 0.15 H_2O (383.89): calcd. (%): C 56.66, H 7.13, N 11.02; found (%): C 56.27, H 7.08, N 10.94; MS (HR-MS): $C_{16}H_{23}N_2O_6$ $[M+H]^+$, calcd.: m/z = 382.1987, found: m/z = 382.1992.

2-N-benzyloxycarbonyl-1-tert.-butoxycarbonyl-1,2 (S)-diamino-tridecan-3-ol (9)

The amide **7** (3.6 g, 9.5 mmol) was dissolved under argon in 100 ml absolute THF and cooled to -50°C. To this mixture 47.5 ml of 1 M solution of decylmagnesium bromide in diethyl ether (47.5 mmol) was added dropwise. After the reaction was completed (tlc analysis, petroleum ether/ethyl acetate 5 : 1), the mixture was poured onto 50 ml of 1 M NaH_2PO_4 while stirring vigorously, and extracted with ethyl acetate (3 x 50 ml). The combined organic extracts were washed subsequently with 1 M NaH_2PO_4 (1 x 30 ml) and brine (1 x 30 ml), dried over $MgSO_4$, filtered and evaporated in vacuo. The residue was dissolved in 100 ml isopropyl alcohol and cooled to 0°C, sodium borohydride (1.07 g, 28.5 mmol) was added and the mixture was stirred for 12 h at 0°C. The reaction was quenched by dropwise addition of 120 ml 1 N HCl. The resulting solution was extracted with ethyl acetate (3 x 50 ml) and the combined organic phases were washed with saturated $NaHCO_3$-solution (2 x 30 ml) and brine (1 x 30 ml), dried over $MgSO_4$, filtered and evaporated in vacuo. The residue was chromatographed on silica gel (petrol ether/ethyl acetate 5 : 1, R_f = 0.32) yielding 2.03 g (46%) of the diastereomeric alcohols **9** as white powder (m. p. 186°C). As indicated by the integral ratio of the two signals of the 3-OH group in the ^{1}H NMR spectrum, **9** occurs as 3 : 1 mixture of the diastereomers.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.85 (t, J = 6.6 Hz, 3 H, CH_3); 1.12-1.56 (m, 27 H, Alkyl-CH_2, C(CH_3)$_3$); 3.05 and 3.16 (d, br, J = 6 Hz, 1 H, diastereomeric O*H*, integration ratio 3 : 1); 3.32 (m, 1 H, CH-NH); 3.46-3.57 (m, 2 H, CH_2-CH); 3.62 (m, 1 H, CH-OH); 4.99 (m, 1 H, N*H*COOC(CH_3)$_3$), 5.06 (s, 2 H, O-CH_2-C_6H_5); 5.33 (d, br., J = 8.4 Hz, 1 H, N*H*COOCH_2C$_6$H$_5$); 7.31 (m, 5 H, C_6H_5).

^{13}C NMR (62.89 MHz, $CDCl_3$), values from the major diastereomer: δ = 14.78 (CH_3); 19.21, 21.95, 21.45, 22.39, 22.58, 23.34, 26.70, 28.97, 29.99, 30.27, 32.57 (C(CH_3)$_3$, Alkyl-CH_2); 40.87 (CH_2-CHOH); 53.64 (C(CH_3)$_3$); 56.52 (CH-NH); 67.54(CH-OH); 72.93 (CH_2-NH); 80.75 (O-CH_2), 129.20 (C_6H_5); 130.21, 131.62, 137.02 (C_6H_5); 157.09 (NH$COOC$(CH_3)$_3$); 170.39 (NH$COOCH_2C_6H_5$).

Analysis: $C_{26}H_{44}N_2O_5$ x 0.15 H_2O (467.026): calcd. (%): C 66.89, H 9.49, N 5.99; found (%): C 67.20, H 9.76, N 5.62; MS (FAB-MS): $C_{26}H_{45}N_2O_5$ $[M+H]^+$, calcd.: m/z = 465.332, found: m/z = 465.20.

4-tert.-Butoxycarbonylaminomethyl-5-decyl-oxazolidin-2-one (10)

The diprotected amino alcohols **9** (150 mg, 0.3 mmol) were dissolved in methanol and palladium on charcoal (10%; caution: pyrophoric) was added. The mixture was stirred under 1 atm of H_2 for 12 h. After the reaction was completed (tlc analysis petrol ether/ethyl acetate 5 : 1), the catalyst was filtered off and the methanol was removed under reduced pressure to yield 95 mg (95%) of the deprotected amines as colourless oil. The crude amino alcohols were dissolved in dichloromethane (10 ml/mmol) and triphosgene (24.7 mg, 0.08 mmol) and triethylamine (131 µl, 0.83 mmol) were added. After 1 h the reaction mixture was hydrolyzed with water. The organic layer was separated and washed successively with saturated sodium hydrogencarbonate solution and brine, dried over $MgSO_4$, filtered off and evaporated in vacuo. The residue was chromatographed on silica gel with petrol ether/ ethyl acetate = 1 : 1 as eluent. **10** was obtained as a colourless oil, R_f = 0.42, yield = 68 mg (67 %).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.81 (t, J = 6.89 Hz, 3 H, C*H*$_3$); 1.12-1.42 (m, 25 H, Alkyl-C*H*$_2$, C(C*H*$_3$)$_3$); 1.81-2.13 (m, 2 H, C*H*$_2$-CH); 3.34 dd, J = 8.39 Hz, J = 9.35 Hz, 1 H, C*H*$_2$-CHN); 3.37 (dd, J = 8.21 Hz, J = 9.34 Hz, 1 H, C*H*$_2$-CHN); 3.62 (ddd, J = 8.40 Hz, J = 3.6 Hz, $J_{4,5}$ = 7.92 Hz,1 H, 4-H, C*H*-N), values from the *erythro*-diastereomer; 3.81 (m, 1 H, C*H*-O); 4.69 (m, br, 1 H, N*H*).

O,O-Diethyl-N-[2 (S)-benzyloxycarbonylamino-3-hydroxy-tridecyl]-phosphoramide (12)

The diastereomeric amino alcohols **9** (452 mg, 0.967 mmol) were dissolved in methanol saturated with hydrogen chloride (20 ml) and stirred for 12 h at ambient temperature. After the reaction was complete (tlc analysis, petrol ether/ethyl acetate 5 : 1), the aequous solution was cooled to 0°C on an ice-water bath and neutralized with a saturated $NaHCO_3$ solution to pH = 7-8. The crude amines **11** were extracted with dichloromethane (3 x 10 ml) and the combined organic extracts were dried over $MgSO_4$, filtered and concentrated under reduced pressure to give 364 mg of crude amines **11** as white powder.

For the further reaction, the crude amines **11** (364 mg, 0.93 mmol) and triethylamine (125 µl, 0.95 mmol) were dissolved in 30 ml chloroform and cooled to 0°C. To this mixture 153 µl (0.96 mmol) of phosphoric acid diethylester chloride were added dropwise and stirring was continued for 12 h at ambient temperature. Volatile components were distilled off and the remaining residue was chromatographed on silica gel with dichloromethane/methanol = 30 : 1 as eluent. The phosphoramides **12** were obtained as white powder, m. p. 186°C, R_f (dichloromethane/methanol = 30 : 1) = 0.42, yield = 312 mg (65.6%) as 3 : 1 mixture of diastereomers. The diastereomeric ratio was estimated by the integration ratio of the 3-OH signal.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.86 (t, J = 6.54 Hz, 3 H, C*H*$_3$); 1.17-1.32 (m, 24 H, Alkyl-C*H*$_2$, P(O-CH$_2$C*H*$_3$)$_2$; 1.41-1.53 (m, 2 H, C*H*$_2$-CH); 2.94 and 3.02 (d, br, J = 5.8 Hz, 1 H, diastereomeric O*H*, integration ratio 3 : 1; 3.19-3.69 (m, 5 H, C*H*$_2$-NH, C*H*-NH, C*H*-OH, N*H*COOC(CH$_3$)$_3$); 3.98 (q, J = 7 Hz, 4 H, P(OC*H*$_2$CH$_3$)$_2$); 5.07 (s, 2 H, C*H*$_2$-C$_6$H$_5$); 5.80 (d, br, J = 8 Hz, 1 H, N*H*COOCH$_2$C$_6$H$_5$); 7.32 (m, 5 H, C$_6$*H*$_5$).

^{13}C NMR (62.89 MHz, $CDCl_3$), values from the major diastereomer: δ = 14.14 (*C*H$_3$); 16.05, 16.16 (P(OCH$_2$*C*H$_3$)$_2$); 22.70, 26.15. 29.38, 29.66, 31.92, 33.07, 33.14, 33.91 (Alkyl-*C*H$_2$); 41.21 (*C*H$_2$CHOH); 55.32 (*C*HNH); 62.53, 62.76 (P(O*C*H$_2$CH$_3$)$_2$); 67.11 (*C*HOH); 72.38 (*C*H$_2$NH); 82.25 (O*C*H$_2$C$_6$H$_5$); 128.06, 128.35, 132,56, 136.59 (*C*$_6$H$_5$); 156.55 (*C*OOCH$_2$C$_6$H$_5$).

^{31}P NMR (121.49 MHz, $CDCl_3$): δ = 9.17.

Analysis: $C_{25}H_{45}N_2O_6P$ (500.301): calcd. (%): C 59.98, H 9.06, N 5.60; found (%): C 60.02, H 9.25, N 5.72; MS (FAB-MS): $C_{25}H_{46}N_2O_6P$ $[M+H]^+$, calcd.: m/z = 501.301, found: m/z = 501.42.

O,O-Diethyl-N-[2(S)-amino-3-hydroxy-tridecyl]-phosphoramide (13)

The phosphoramides **12** (200 mg, 0.4 mmol) were dissolved in methanol and palladium on charcoal (10%; caution: pyrophoric) was added. The mixture was stirred under 1 atm of H_2 for 12 h. After the reaction was completed (tlc analysis dichloromethane/methanol 30 : 1), the catalyst was filtered off and the methanol was removed under reduced pressure to yield 135 mg (95%) of **13** as colourless oil (R_f (dichloromethane/methanol/1% ammonia 10 : 1) = 0.23). As indicated by the integral ratio of the two signals of the 3-OH group in the ^{1}H NMR spectrum, **13** occurs as 3 : 1 mixture of the diastereomers.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.85 (t, J = 7 Hz, 3 H, C*H*$_3$); 1.18-1.26 (m, 16 H, Alkyl-C*H*$_2$); 1.29 (t, J = 7.1 Hz, 6 H, P(OCH$_2$C*H*$_3$)$_2$); 1.41-1.46 (m, 2 H, C*H*$_2$-CHOH); 2.89 and 3.04 (d, br, J = 6.2 Hz, 1 H, diastereomeric OH, integration ratio 3 : 1); 3.10-3.47 (m, 4 H, C*H*$_2$-NH, N*H*$_2$); 3.57-3.85 (m, 2 H, C*H*-NH, C*H*-OH); 4.04 (dq, J = 7.1 Hz, $^3J_{HP}$ = 2.75 Hz, P(OC*H*$_2$CH$_3$)$_2$).

^{13}C NMR (62.89 MHz, $CDCl_3$), values from the major diastereomer: δ = 14.32 (*C*H$_3$); 16.26, 16.32 (P(OCH$_2$*C*H$_3$)$_2$); 22.73, 24.64, 25.92, 26.32, 29.55, 31.8, 33.07, 33.53 (Alkyl-*C*H$_2$); 42.48 (*C*H$_2$CHOH); 56.42 (*C*HNH$_2$); 62.68, 62.83 (P(O*C*H$_2$CH$_3$)$_2$); 67.31 (*C*HOH); 72.56 (*C*H$_2$NH).

^{31}P NMR (121.49 MHz, $CDCl_3$): δ = 9.21.

Analysis: $C_{17}H_{39}N_2O_4P$ (366.480): calcd. (%): C 55.72, H 10.73, N 7.64; found (%): C 55.36, H 10.44, N 7.31; MS (FAB-MS): $C_{17}H_{40}N_2O_4P$ $[M+H]^+$, calcd.: m/z = 367.47, found: m/z = 367.40.

O,O-Dibenzyl-N-[2 (S)-benzyloxycarbonylamino-3-hydroxy-tridecyl]-phosphoramide (14)

The diastereomeric amino alcohols **9** (460 mg, 0.98 mmol) were dissolved in methanol saturated with hydrogen chloride (20 ml) and stirred for 12 h at ambient temperature. After the reaction was complete (tlc analysis, petrol ether/ethyl acetate 4 : 1), the aqueous solution was cooled to 0°C on an ice-water bath and neutralized with a saturated $NaHCO_3$ solution to pH = 7-8. The crude amines **11** were extracted with dichloromethane (3 x 10 ml) and the combined organic extracts were dried over $MgSO_4$, filtered and concentrated under reduced pressure to give 380 mg of crude amines **11** as white powder.

For the further reaction, the crude amines **11** (380 mg, 0.96 mmol) were dissolved under argon in 5ml tetrachloromethane. To this solution 115 µl (0.52 mmol) of dibenzylphosphite were added dropwise and the mixture was stirred for 12 h at ambient temperature. The solvent was concentrated under reduced pressure and the remaining residue was chromatographed on silica gel with dichloromethane/methanol 40 : 1 to yield 442 mg (68%) of **14** as white powder, R_f (dichloromethane/methanol 40 : 1) = 0.27.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.86 (t, J = 6.43 Hz, 3 H, C*H*$_3$); 1.14-1.48 (m, 18 H, Alkyl-C*H*$_2$); 2.87 and 2.98 (d, br, 1 H, diastereomeric OH, integration ratio 3 : 1); 3.02-3.30 (m, 3 H, N*H*, C*H*$_2$NH); 3.41-3.68 (m, 2 H, C*H*-NH, C*H*-OH); 4.96 (s, 2 H, O-C*H*$_2$-C$_6$H$_5$); 5.00 (s, 2 H, O-C*H*$_2$-C$_6$H$_5$); 5.04 (s, 2 H, COO-C*H*$_2$-C$_6$H$_5$);

5.45 (d, br, J = 7.72 Hz, 1 H, N*H*COOCH$_2$C$_6$H$_5$); 7.29 (s, 10 H, P(OCH$_2$-C$_6$*H*$_5$)$_2$); 7.32 (m, 5 H, COOCH$_2$C$_6$*H*$_5$).

^{13}C NMR (62.89 MHz, $CDCl_3$), values from the major diastereomer: δ = 14.34 (*C*H$_3$); 22.70, 24.6, 26.15. 29.38, 29.66, 31.92, 33.14, 33.91 (Alkyl-*C*H$_2$); 41.21 (*C*H$_2$CHOH); 55.32 (*C*HNH); 67.11 (*C*HOH); 72.38 (*C*H$_2$NH); 82.25 (O*C*H$_2$C$_6$H$_5$); 84.35, 85.21 (P(O*C*H$_2$C$_6$H$_5$)$_2$); 128.12, 128.35, 128.42, 128.47, 129.43, 130.23, 131.91, 132.12, 132.56, 135.67, 136.34, 136.59 (P(OCH$_2$*C*$_6$H$_5$)$_2$), *C*OOCH$_2$C$_6$H$_5$); 156.55 (*C*OOCH$_2$C$_6$H$_5$).

^{31}P NMR (121.49 MHz, $CDCl_3$): δ = 9.73.

Analysis: $C_{35}H_{49}N_2O_6P$ (624.756): calcd. (%): C 67.29, H 7.91, N 4.48; found (%): C 67.29, H 8.02, N 7.89; MS (FAB-MS): $C_{35}H_{50}N_2O_6P$ $[M+H]^+$, calcd.: m/z = 625.34, found: m/z = 625.40.

N-[2 (S)-amino-3-hydroxy-tridecyl]-phosphoramide (15)

The phosphoramide dibenzylester **14** (256 mg, 0.41 mmol) was dissolved in methanol and palladium on charcoal (10%; caution = pyrophoric) was added. The reaction mixture was stirred under 1 atm of H_2 for 12 h. After the reaction was completed (tlc analysis, dichloromethane/methanol 30 : 1), the catalyst was filtered off and the methanol was removed under reduced pressure to yield 122 mg (96%) of **15** as colourless oil (R_f (chloroform/methanol/water 60 : 35 : 8) = 0.29) as 3 : 1 mixture of diastereomers. The diastereomeric ratio was estimated by the integration ratio of the 3-OH signal.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.81 (t, J = 6.23 Hz, 3 H, C*H*$_3$); 1.16-1.52 (m, 16 H, Alkyl-C*H*$_2$); 2.50 and 2.68 (d, br, J = 5.7 Hz, 1 H, diastereomeric OH, integration ratio 3 : 1); 2.63-2.96 (m, 2 H, C*H*$_2$-OH); 3.01-3.32 (m, 1 H, C*H*-NH$_2$); 3.43-3.68 (m, 2 H, C*H*$_2$-NH); 3.82-3.98 (m, 1 H, C*H*-OH); 5.4-6.6 (s, br, 4 H, P(O*H*)$_2$, N*H*$_2$).

^{13}C NMR (62.89 MHz, $CDCl_3$), values from the major diastereomer: δ = 14.23 (*C*H$_3$); 21.72, 23.6, 25.35. 28.38, 28.76, 30.72, 33.54, 33.81 (Alkyl-*C*H$_2$); 42.21 (*C*H$_2$CHOH); 55.62 (*C*HNH$_2$); 67.51 (*C*HOH); 73.45 (*C*H$_2$NH).

Analysis: $C_{13}H_{31}N_2O_4P$ x 0.3 H_2O (315.426) calcd. (%): C 49.45, H 10.09, N 8.87; found (%): C 49.12, H 10.12, N 8.67 MS (FAB-MS): $C_{13}H_{32}N_2O_4P$ $[M+H]^+$, calcd.: m/z = 311.209, found: m/z = 311.30.

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