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Synthesis of Phosphonate Analogues of Sphinganine-1-phosphate and Sphingosine-1-phosphate

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Abstract: Phosphonic acid derivatives **14** and **18** were prepared as analogues of sphinganine-1-phosphate **(4)** and sphingosine-1-phosphate **(3)**. Key steps of the synthesis are the highly face-selective conjugate addition of the lithium salt of Schöllkopf's bislactim ether **5** to the diethyl vinylphosphonate **7** and the alkylation of the amino aldehyde **11** with metal organic reagents.

Long chain bases as sphingosine **1** and sphinganine **2** occur in components of cellular membranes, e.g. ceramide, sphingomyelin, cerebrosides and gangliosides [1]. 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 [2].

The degradation of long chain bases is reported to require ATP-dependent phosphorylation at the 1-position leading to the 1-phosphates of sphingosine **3** and sphinganine **4** (fig. 1). This phosphorylation step is catalyzed by the cytosolic enzyme sphingosine kinase [3,4]. The 1-phosphates **3** and **4** are cleaved by the action of a pyridoxal-phosphate dependent lyase to yield a long chain aldehyde and ethanolamine phosphate [5].

In additon to their role as catabolic intermediates, recent reports suggest further roles of sphingosine **1** and sphingosine-1-phosphate **3**. Sphingosine was found to be a potent inhibitor of protein-kinase C both in vitro and in vivo [6]. In addition, it was demonstrated that low concentrations of sphingosine stimulate the proliferation of quiescent Swiss 3T3 fibroblasts in a fundamentally different, protein kinase C independent pathway [7].

Sphingosine-1-phosphate is reported to be a potent mitogen in Swiss 3T3 fibroblasts [3,8] and to stimulate the release of calcium from internal sources [9]. Furthermore, activation of sphingosine kinase and formation of sphingosine-1-phosphate are supposed to be important events in the signal transduction cascade initiated by the mitogens PDGF (platelet-derived growth factor) and FCS (fetal calf serum) [10].

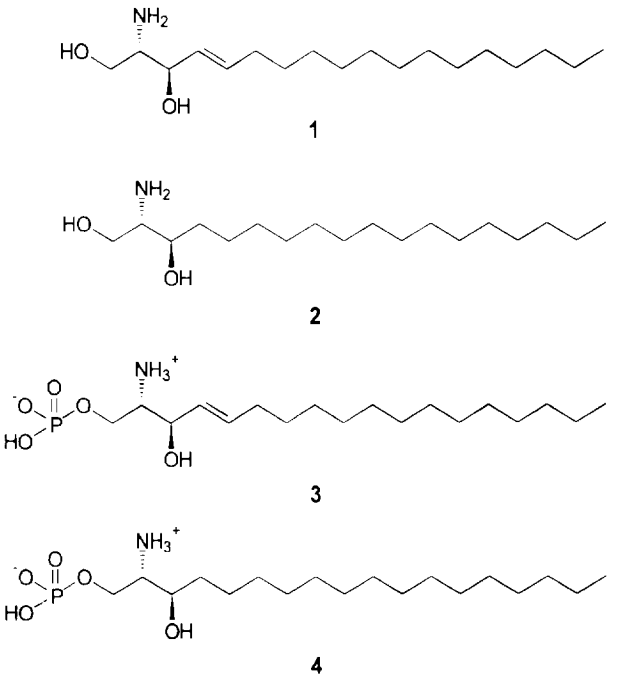


Figure 1.

Recently, it was demonstrated that sphingosine-1-phosphate inhibits motility of melanoma cells at very low concentrations, at which sphingosine shows no inhibitory effect [11,12]. These findings suggest that sphingosine-1-phosphate may have antimetastatic and inflammatory properties.

Finally, sphingosine-1-phosphate elevates phosphatidic acid levels [13] and activates DNA binding activity of AP-1 [14] which may link sphingolipid metabolism pathways to *ras*-mediated signaling pathways. Sphingosine-1-phosphate decreases cellular cAMP levels and also causes a drastic decrease in isoproterenol- and forskolin-stimulated cAMP accumulation [15]

Therefore, sphingosine-1-phosphate **(3)** may act as an intracellular lipid second messenger which is involved in calcium release and the regulation of the cell growth induced by sphingosine **(1)**.

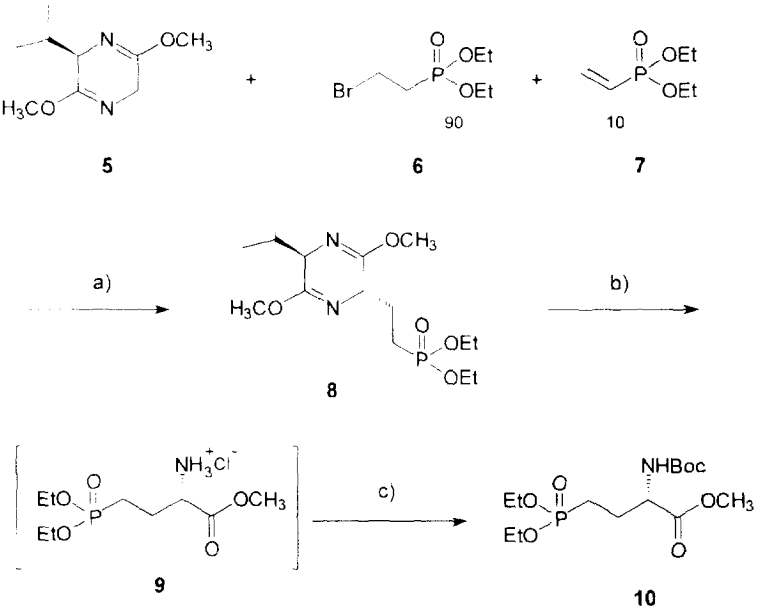
To clarify the function of **3** and **4** we required analogoues compounds with improved metabolic stability.

In this paper we describe the synthesis of **14** and **18** as structural analogues of shingosine-1-phosphate and sphinganine-1-phosphate modified in the headgroup. Compared to the natural sphingosine-1-phosphate and sphinganine-1-phosphate the P-O bond of the phosphonic acid ester at the 1 position is substituted by an P-C - bond, which is resistent to the action of phosphohydrolases. In the early 70th Stoffel et al. [16] described the synthesis of 1-deoxysphinganine-1-phosphonate. In contrast to the natural sphinganine-1-phosphate **(4)**, the $CH_2OPO_3^{2-}$ group is substituted by an $CH_2PO_3^{2-}$ group which reduces the length of the molecule by approximately 1.32 Å. This phosphonate is highly toxic when adminstered intravenously to rats in amounts between 2 and 10 µmoles and furthermore with human blood hemolysis occurs even in concentrations of 2 x

10^{-5} M. 1-Deoxy-sphinganine-1-phosphonate is both a substrate and an inhibitor for the sphingosine-1-phosphate lyase. Michaelis-Menten constants of the 1-deoxy-phosphonates and of the 1-phosphate were found to be closely identical at 1.6 x 10^{-5} M. However, cleavage of 1-deoxy-sphinganine-1-phosphonate occurred at 10-fold slower rate as that of sphinganine-1-phosphate. 1-Deoxysphinganine-1-phosphonate is a competitve inhibitor of the sphinganine-1-phosphate lyase with a K_i value of 5 x 10^{-6} M.

In order to imitate **3** and **4** more closely, we started with the synthesis of the corresponding sphinganine- and sphingosine-1-phosphonates (**14**, **18**), in which the $CH_2OPO_3^{2-}$ group is replaced by a $CH_2CH_2PO_3^{2-}$ group.

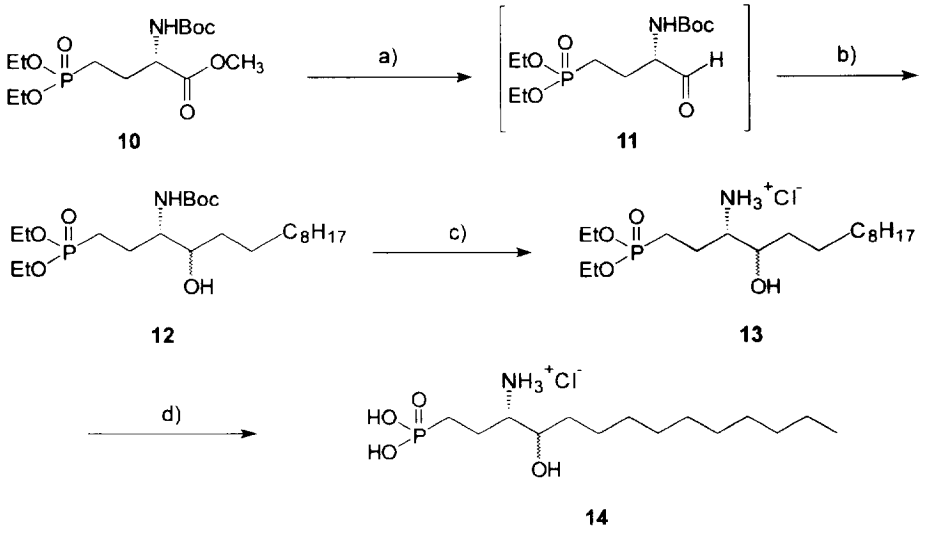
Starting with the bislactimether **5** of cyclo (-L-Val-Gly), [17] we prepared compound **8** by alkylation with an equimolar solution of diethyl 2-bromoethylphosphonate (**6**) containing 10% of the diethyl vinylphosphonate **7** (scheme 1). These conditions are reported to give only exclusively the diastereomer **8** in good yield [18]. Presumably the addition of the lithium salt of **5** to **7** is much faster than the alkylation with **6**. The Michael adduct is then trapped by **6** in a dehydrohalogenation reaction regenerating the reactive species **7**. Only one diastereomer was detectable in the ^{1}H NMR- and ^{13}C NMR spectrum.



Scheme 1 a) n-BuLi, THF, 81%; b) 0.25 N HCl, THF/acetonitrile; c) Boc_2O, dioxane/water, 74%.

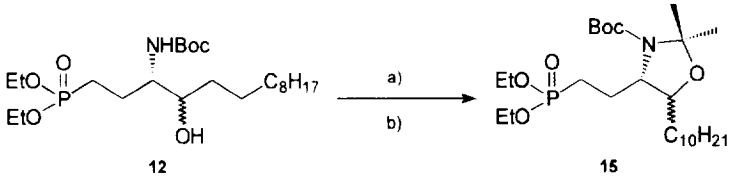
Mild acid hydrolysis of the bislactim ether **8** afforded the amino ester hydrochloride **9**, which was converted without further purification to the corresponding N-Boc derivative **10** in good yield. Reduction of **10** with diisobutylaluminium hydride [19] in toluene yielded the amino aldehyde **11** (scheme 2). Since α-amino aldehydes are reported to be configurationally instable [20], **11** was used for alkylation without further purification or

storage. Alkylation with decylmagnesium bromide following a procedure previously reported [21] afforded the diastereomeric alcohols **12** as 3 : 1 mixture.



Scheme 2: a) diisobutylaluminium hydride, toluene; b) decylmagnesium bromide, THF, 45%; c) MeOH/HCl, 82%; d) 4 N HCl, 76%

The relative configuration of the alcohols **12** was established after transformation into the corresponding oxazolidines **15** [22] (scheme 3). According to ^{1}H NMR investigations of **15**, the major diastereomer is *threo*-configurated.

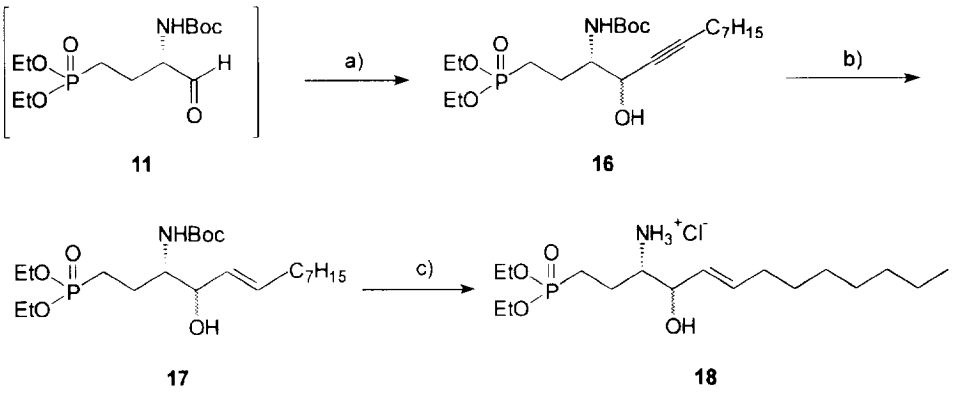


Scheme 3: a) MeOH/HCl, 82%; b) pyridinium-toluene-4-sulfonate, dichloromethane, 2,2-dimethoxypropane, 86%

The enantiomeric purity of the alcohols **12** were assured by the *Mosher* method [23]. The ^{1}H NMR spectrum of the corresponding Mosher derivative of **12** is consistent with a racemization degree minor than 10% (data not shown).

Removal of the Boc group in **12** with HCl gas in methanol afforded the hydrochlorides **13** in excellent yield. The phosphonic acid diethylester was converted into the corresponding phosphonic acid **14** by treatment with 4 N HCl in 76% yield.

The synthesis of **18** started with the additon of the lithium salt of 1-nonine to the aldehyde **11** following a procedure previously reported [24] (scheme 4). The alkylation of amino aldehyde **11** afforded **16** as 2 : 1 mixture of diastereoisomers.



11 **16** **17** **18**

Scheme 4: a) 1-nonine, THF, 43%, b) lithium, ammonia, 86%; c) MeOH/HCl, 85%

Treatment of **16** with lithium in ammonia afforded the trans-configurated derivative **17** [25]. Deprotection of the diastereomeric amino alcohols **17** with a saturated methanolic solution of hydrogen chloride afforded **18** as 2 : 1 mixture of diastereomers. All attempts to hydrolyze the phosphonic acid diethylester of **18** with 4 N HCl led to the decomposition of the hydrochlorides **18**.

The relative configuration of amino alcohols **17** was estabilished by reduction of **17** with hydrogen and Pd-C in methanol. ^{1}H NMR analysis of the corresponding amino alcohols and comparison with the ^{1}H NMR spectrum of **12** suggest that the alkylation of the aldehyde predominantly leads to the *threo*-isomer (data not shown). The enantiomeric purity of the alcohols **17** was assured by the *Mosher* method [22]. The ^{1}H NMR spectrum of the corresponding Mosher derivative of **17** is consistent with a racemization degree minor than 10% (data not shown).

Biochemical properties of the synthesized compounds will be reported elsewhere [26].

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, 0.063-0.200 mm). 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 analysis were performed at the Institute of Organic Chemistry and Biochemistry, Bonn, Microanalytical Department.

(2S, 5R)-2-[2-(Diethoxyphosphoryl)ethyl]-2,5-dihydro-5-isopropyl-3,6-dimethoxypyrazine (8)

The bislactim ether **5** (1.00 g, 5.4 mmol) was dissolved under argon atmosphere in 20 ml dry THF and cooled to -78°C. A 1.6 M solution of butyllithium in hexane (3.5 ml, 5.6 mmol) was injected and the stirring was continued for 30 min. A solution of **6** (1.23 ml, 5.09 mmol) containing 10% **7** (87.22 µl, 0.559 mmol) in 5 ml dry THF was added slowly and the mixture was stirred for 2 h at -78°C. After addition of acetic acid (320 µl, 5.5 mmol) in 1 ml THF the reaction mixture was allowed to warm up to room temperature. The solvent was evaporated under reduced pressure and the residue was portioned between 5 ml water and 20 ml diethyl ether. The aqueous layer was separated and extracted with diethyl ether (3 x 10 ml). The combined organic extracts were dried over $MgSO_4$ and evaporated under reduced pressure. The crude compound **8** was purified by bulb-to-bulb distillation. Yield: 1.52 g (81%) **8** as colourless oil, b.p. 165°C, 0.15 Torr; R_f (dichloromethane/methanol = 40:1) = 0.36. Only one diastereomer was detectable in the ^1H NMR and ^{13}C NMR spectra.

^1H NMR (250 MHz, $CDCl_3$): δ = 0.69, 1.01 (2 d, J = 7 Hz, 6 H, $CH(CH_3)_2$); 1.31 (t, J = 7 Hz, 6 H, $P(OCH_2CH_3)_2$); 1.60 - 2.29 (m, 5 H, CH_2CH_2P, $CH(CH_3)_2$); 3.65, 3.68 (2 s, 6 H, OCH_3); 3.94 (dd, ^3J = 3 Hz, 1 H, 5-H); 4.02 (m, 1 H, 2-H); 4.12 (dq, $^3J_{HH}$ = 7 Hz, $^3J_{HP}$ = 7 Hz, 4 H, $P(OCH_2CH_3)_2$).

^{13}C NMR (62.89 MHz, $CDCl_3$): δ = 17.00, 17.09, 17.89, 19.62 ($P(OCH_2CH_3)_2$, $CH(CH_3)_2$); 22.76, 27.78 (CH_2CH_2P); 32.59 ($CH(CH_3)_2$); 53.13, 53.06 (OCH_3); 61.64, 62.08, 62.17 (C-5, $P(OCH_2CH_3)_2$); 163.54, 164.92 (C=N).

^{31}P NMR (121.49 MHz, $CDCl_3$): δ = 32.14.

Analysis: $C_{15}H_{29}N_2O_5P$ (348.378) calcd. (%): C 51.72, H 8.39, N 8.04; found (%): C 51.95, H 8.61, N 8.55; MS (70 eV): $C_{15}H_{29}N_2O_5P$ $[M]^+$, calcd.: m/z = 348.1814, found: m/z = 348.1827.

Methyl (S)-2-(N-tert. butoxycarbonyl)-amino-4-(diethoxyphosphoryl)butanoate (10)

Compound **8** (1.00 g, 2.9 mmol) was suspended in a mixture of THF/acetonitrile 3:1 (4 ml) and 0.25 N HCl (92 ml, 23 mmol) and stirred at room temperature for 3 h. The mixture was extracted once with diethyl ether which was discarded. After evaporation of the water under reduced pressure, the residue was supplied for the next preparation without further purification.

The residue (crude **9**) was dissolved in dioxane/water 1 : 1 (50 ml), triethylamine (1.54 ml, 11.6 mmol) and di-tert.-butyloxycarbonyl dicarbonate (1.38 g, 6.09 mmol) was added and stirring was continued for 6 h at ambient temperature. After extraction of the aqueous layer with dichloromethane (3 x 20 ml), the combined organic layers were washed with brine (1 x 30 ml), dried over $MgSO_4$ and concentrated under reduced pressure. The remaining oil was chromatographed on silica gel (dichloromethane/methanol 40 : 1) yielding 372 mg (74%) **11** as an yellow oil, b.p. 155°C, 0.5 Torr, R_f (dichloromethane/methanol = 40:1) = 0.30.

^1H NMR (250 MHz, $CDCl_3$): δ = 1.29 (t, J = 7 Hz, 6 H, $P(OCH_2CH_3)_2$); 1.41 (s, 9 H, $C(CH_3)_3$); 1.63-2.19 (m, 4 H, CH_2CH_2P); 3.72 (s, 3 H, OCH_3); 4.06 (dq, $^3J_{HH}$ = 7 Hz, $^3J_{HP}$ = 4 Hz, 4 H, $P(OCH_2CH_3)_2$); 4.30 (m, 1 H, 2-H); 5.13 (d, J = 9 Hz, br, 1 H, NH).

^{13}C NMR (62.89 MHz, $CDCl_3$): δ = 17.05, 17.14, ($P(OCH_2\underline{C}H_3)_2$); 21.37, 23.64 ($\underline{C}H_2CH_2P$); 26.59, 26.66, 28.94 ($C(\underline{C}H_3)_3$); 53.16 ($\underline{C}(CH_3)_3$), 54.04, 54.38 (CHNH, OCH_3); 62.38, 62.45 ($P(O\underline{C}H_2CH_3)_2$); 156.00 ($\underline{C}OOC(CH_3)_3$); 173.04 ($\underline{C}OOCH_3$).

^{31}P NMR (121.49 MHz, $CDCl_3$): δ = 30.19.

Analysis: $C_{14}H_{28}NO_7P$ x 0.25 H_2O (357.67) calcd. (%): C 46.93, H 8.03, N 4.18; found (%): C 46.97, H 7.56, N 3.92; MS (70 eV): $C_{14}H_{29}NO_7P$ $[M+H]^+$, calcd.: m/z = 354.1681, found: m/z = 354.1674.

Diethyl-3-(S)-tert.-butoxycarbonylamino-4-hydroxy-tetradecyl-1-phosphonate (12)

Methylester **10** (2.19 g, 6.2 mmol) was dissolved in 20 ml dry toluene and cooled to -78°C. To this cooled solution a solution of 1.5 M diisobutylaluminium hydride in toluene (7.23 ml, 10.85 mmol) was added.. The rate of addition was adjusted so as to keep the bath temperature below -70°C and takes approximately 1 h to complete. After stirring for additional 2 h at -78°C, tlc analysis (dichloromethane/methanol 30 : 1) shows the formation of the desired aldehyde **11**. The reaction was quenched by slowly adding 1.3 ml methanol. The temperature of the ice bath again was kept below -70°C. The resulting white suspension was slowly poured into 22 ml of ice cold 1 N hydrochloric acid and stirred for additional 15 min. After extraction of the aqueous layer with ethyl acetate (3 x 40 ml), the combined organic layers were washed with brine (1 x 20 ml), dried over $MgSO_4$ and concentrated under reduced pressure to give 1.62 g of crude product **11** (aldehyde) as an colourless oil.

For the further reaction, the crude aldehyde **11** (1.62 g, 4.96 mmol) was dissolved in 40 ml dry THF under argon and cooled to -78°C. To this mixture 15 ml of 1 M solution of decylmagnesium bromide in diethyl ether (15 mmol) was added dropwise. After the reaction was completed (tlc analysis dichloromethane/methanol 30 : 1), the reaction was quenched by adding 50 ml of saturated aqueous ammonium chloride solution and the mixture was allowed to warm up to room temperature. The resulting solution was extracted with ethyl acetate (3 x 40 ml) and the combined organic extracts were dried over $MgSO_4$ and evaporated under reduced pressure. The residue was chromatographed on silica gel (dichloromethane/methanol 30 : 1) to yield 1.04 g (45%) of the diastereomeric alcohols **12** as yellow oil (R_f dichloromethane/methanol = 30 : 1) = 0.26). As indicated by the integral ratio of the two signals of the 4-OH group in the ^{1}H NMR spectrum, **12** occurs as 3 : 1 mixture of the diastereomers.

^{1}H NMR (250 MHz, $CDCl_3$): δ = 0.86 (t, J = 3 Hz, 3 H, CH_3); 1.24-1.42 (m, 25 H, $C(CH_3)_3$, Alkyl-CH_2); 1.31 (t, J = 7 Hz, 6 H, $P(OCH_2CH_3)_2$); 1.57-2.01 (m, 6 H, CH_2CH_2P, CH_2CHOH); 2.37 und 2.56 (d, br, 1 H, diastereomeric OH, integration ratio 3 : 1); 3.41-3.62 (m, 2 H, CHOH, CHNH); 4.07 (dq, $^3J_{HH}$ = 7 Hz, $^3J_{HP}$ = 4 Hz, 4 H, $P(OCH_2CH_3)_2$); 4.88 (br, d, J = 9 Hz, 1 H, NH).

^{13}C NMR (62.89 MHz, $CDCl_3$), values from the major diastereomer: δ = 13.60 (CH_3); 15.96, 16.02 ($P(OCH_2\underline{C}H_3)_2$); 19.21, 21.95, 21.45, 22.39, 22.58, 24.97, 25.68, 26.39, 26.86, 28.73, 29.37, 29.57, ($C(\underline{C}H_3)_3$, Alkyl-CH_2); 31.87, 32.17 ($CH_2\underline{C}H_2P$), 53.34 ($\underline{C}(CH_3)_3$), 55.70 (HNCH); 62.90, 62.99 ($P(O\underline{C}H_2CH_3)_2$); 69.66, ($CH_2\underline{C}HOH$); 157.09 ($\underline{C}OOC(CH_3)_3$).

^{31}P NMR (121.49 MHz, $CDCl_3$): δ = 32.13.

Analysis: $C_{23}H_{48}NO_6P$ x 0.25 H_2O (469.823) calcd. (%): C 58.70, H 10.40, N 2.97; found (%): C 58.58, H 10.45, N 3.23; MS (FAB-MS): $C_{23}H_{49}NO_6P$ $[M+H]^+$, calcd.: m/z = 466.321, found: m/z = 466.30.

Diethyl-3-(S)-amino-4-hydroxy-tetradecyl-1-phosphonate hydrochloride (13)

The diastereomeric amino alcohols **12** (130 mg, 0.28 mmol) were dissolved in methanol saturated with hydrogen chloride and stirred for 12 h at ambient temperature. The mixture was extracted once with some methanol which was discarded.

The reaction mixture was evaporated under reduced pressure to yield 92 mg (82%) **13** as a yellow oil.

^{1}H NMR (250 MHz, D_2O): δ = 0.84 (t, J = 7 Hz, 3 H, CH_3); 1.29 (t, J = 7 Hz, 6 H, $P(OCH_2CH_3)_2$); 1.21-1.58 (m, 18 H, Alkyl-CH_2); 1.68-2.11 (m, 4H, CH_2CH_2P), 3.48-3.58 (m, 2 H, $CHNH_3$, $CHOH$); 4.01 (dq, $^3J_{HH}$ = 7 Hz, $^3J_{HP}$ = 4 Hz, 4 H, $P(OCH_2CH_3)_2$).

^{13}C NMR (62.89 MHz, D_2O), values from the major diastereomer: δ = 13.79 (CH_3); 15.86, 15.96 ($P(OCH_2CH_3)_2$); 19.63, 21.85, 24.83, 25.86, 29.25, 29.37, 29.69, 29.76, 31.87, 32.17, 33.05 (CH_2CH_2P, Alkyl-CH_2); 55.70 ($CHNH_3$); 62.92, 63.12 ($P(OCH_2CH_3)_2$); 69.54 (CH_2CHOH).

^{31}P NMR (121.49 MHz, D_2O): δ = 32.06.

Analysis: $ClC_{18}H_{41}NO_4P$ x 0.5 H_2O (410.254) calcd. (%): C 52.91, H 9.66, N 3.63; found (%): C 52.83, H 9.52, N 3.70; MS (FAB-MS): $C_{18}H_{40}NO_4P$ $[M+H-HCl]^+$, calcd.: m/z = 366.277, found: m/z = 366.30.

3-(S)-Amino-4-hydroxy-tetradecyl-1-phosphonic acid hydrochloride (14)

The hydrochlorides **13** (150 mg, 0.376 mmol) were dissolved in 3 ml 4 N HCl and refluxed for 10 h. The solvent was evaporated under reduced pressure and **14** was precipitated as a voluminous white amorphous compound. It was sedimentated by centrifugation and washed several times with some diethyl ether. The residue was dried under reduced pressure (40°C, 12 Torr, 12 h) yielding 86 mg (76%) **14** as white amorphous powder, m.p. about 200°C (dec.), R_f (n-butanol/acetic acid/water = 3 : 1 : 1) = 0.58.

^{1}H NMR (250 MHz, DMSO): δ = 0.84 (t, J = 6.7 Hz, 3 H, CH_3); 1.06-1.49 (m, 18 H,Alkyl-CH_2); 1.52-2.0 (m, 4 H, CH_2CH_2P); 3.46 (m, 1H, $CHNH_3$); 3.86 (m, 1 H, $CHOH$); 7.90 (m, 2 H, $P(OH)_2$).

^{13}C NMR (62.89 MHz, DMSO) values from the major diastereomer: δ = 13.94 (CH_3); 21.46, 21.98, 22.08, 22.61, 22.78, 25.05, 25,48, 29.01, 29.52, 31.17, 31.28 (Alkyl-CH_2, CH_2CH_2P); 41.83 ($CHNH_3$); 65.57 (CH_2CHOH).

^{31}P NMR (121.49 MHz, DMSO): δ = 25.4

Analysis: $ClC_{14}H_{33}NO_4P$ (345.846) calcd. (%): C 48.62, H 9.62, N 4.05; found (%): C 48.61, H 9.73, N 4.29; MS (FAB-MS): $C_{14}H_{33}NO_4P$ $[M+H-HCl]^+$, calcd.: m/z = 310.214, found: m/z = 310.20.

Diethyl-3-tert.-butoxycarbonyl-5-decyl-2,2-dimethyl-oxazolidin-4-(S)-yl-ethyl-phosphonate (15)

The diastereomeric amino alcohols **12** (195 mg, 0.42 mmol) were dissolved in methanol saturated with hydrogen chloride and stirred for 12 h at ambient temperature. The mixture was extracted once with some methanol which was discarded. The solution was neutralized with saturated aqueous ammonium chloride solution and extracted with dichloromethane (3 x 30 ml). The combined organic extracts were dried over $MgSO_4$, filtered and evaporated under reduced pressure.

Pyrdinium-toluene-4-sulfonate (111 mg, 0.42 mmol) was added to a solution of the residue and 2,2-dimethoxypropane (1ml, 8.4 mmol) in dichloromethane (3 ml), and the mixture was stirred at room temperature overnight. The solvents were evaporated under reduced pressure and the residue was purified by Flash chromatography (dichloromethane/methanol 30 : 1, Rf = 0.64) yielding **15** (183 mg, 86%) as a colourless oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.81 (t, J = 3 Hz, 3 H, CH_3); 1.16-1.25 (m, 18 H, , Alkyl-CH_2); 1.26 (t, J = 7 Hz, 6 H, $P(OCH_2CH_3)_2$); 1.40 (s, 9 H, $C(CH_3)_3$); 1.46 (s, 3 H, CH_3); 1.50 (s, 3 H, CH_3); 1.47-1.53 (m, 2 H, CH_2CHOH); 1.60-1.98 (m, 2 H, CH_2CH_2P); 3.69 (ddd, J = 14.6 Hz, J = 9.4 Hz, $J_{3,4}$ = 4.7 Hz, 1 H, CHO); 3.89 (ddd, J = 14.5 Hz, J = 9.6 Hz, $J_{3,4}$ = 4.7 Hz, 1 H, CHN) values from the *threo*-diastereomer; 4.05 (dq, $^3J_{HH}$ = 7 Hz, $^3J_{HP}$ = 4 Hz, 4 H, $P(OCH_2CH_3)_2$).

Diethyl-3-(S)-tert.-butoxycarbonylamino-4-hydroxy-5-tridecinyl-1-phosphonate (16)

To a solution of 1-nonine (2.79 ml, 17.02 mmol) in dry THF (50 ml) was added a 1.6 M solution of n-BuLi (9.67 ml, 15.4 mmol) under argon atmosphere at -23°C The resulting suspension of the lithium alkide was stirred at this temperature for 30 min and then cooled to -78°C. A solution of the crude aldehyde **11** (2.12 g, 6.81 mmol) prepared as described before in dry THF (30 ml) was added dropwise with a syringe. Stirring of the colourless solution was continued for additional 3 h at -78°C. The reaction was quenched by addition of 120 ml of saturated aqueous ammonium chloride solution and the mixture was allowed to warm to room temperature. The resulting solution was extracted with ethyl acetate (3 x 40 ml) and the combinend organic extracts were washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure to give a colourless oil. Purification of the residue by flash chromatography on silica gel with dichloromethane/methanol = 30 : 1 (R_f = 0.28) as eluent afforded 1.32 g (43%) of **16** as 2 : 1 mixture of diastereomers. The diastereomeric ratio was estimated by the integration ratio of the 4-OH signal.

^{1}H NMR (250 MHz, $CDCl_3$): δ = 0.86 (t, J = 6.2 Hz, 3 H, CH_3); 1.19-1.57 (m, 19 H, $C(CH_3)_3$, Alkyl-CH_2); 1.30 (t, J = 7 Hz, 6 H, $P(OCH_2CH_3)_2$); 1.61-2.04 (m, 4 H, PCH_2CH_2); 2.16 (m, 2 H, $CH_2C{\equiv}C$); 2.79 and 2.92, (d, br, 1 H, diastereomeric OH, integration ratio 2 : 1), 3.70 (m, 1 H, CHNH); 4.07 (dq, $^3J_{HH}$ = 7 Hz, $^3J_{HP}$ = 4 Hz, 4 H, $P(OCH_2CH_3)_2$); 4.38 (m, 1 H, CHOH), 4.82 (br, d, J = 9 Hz, 1 H, NH).

^{13}C NMR (62.89 MHz, $CDCl_3$) values from the major diastereomer: δ = 14.74 (CH_3); 17.08, 17.17 ($P(OCH_2CH_3)_2$); 21.82, 21.99, 23.29, 24.07, 25.43, 26.36, 28.53, 29.44, 29.54, 31.06, 31.14 (Alkyl-CH_2, $C(CH_3)_3$, PCH_2CH_2); 56.40 (CHNH), 56.73 ($C(CH_3)_3$); 62.30, 63.41 ($P(OCH_2CH_3)_2$); 65.43 (CHOH); 80.30, 80.56 (C≡C); 156.84 ($COOC(CH_3)_3$).

^{31}P NMR (121.49 MHz, $CDCl_3$): δ = 31.71.

Analysis: $C_{22}H_{42}NO_6P$ (447.274) calcd. (%): C 59.00, H 9.46, N 3.13; found (%): C 58.60, H 9.33, N 3.26; MS (FAB-MS): $C_{22}H_{43}NO_6P$ $[M+H]^+$, calcd.: m/z = 448.282, found: m/z = 448.26.

Diethyl-3-(S)-tert.-butoxycarbonylamino-4-hydroxy-5-tridecenyl-1-phosphonate (17)

Dry ammonia (10 ml) was condensed at -60°C under N_2 atmosphere. To this solution lithium (156 mg, 22.37 mmol) was added in portion and after dissolving of the metal, a solution of **17** (200 mg, 0.447 mmol) in dry

THF (3 ml) was added dropwise. After stirring at -60°C for 12 h, the tlc in ethyl acetate (R_f = 0.26) shows the clean formation of the alkene **17**, at the expense of the starting alkyne. The reaction was quenched at -60°C with 6 g of solid ammonium chloride and the ammonia was allowed to evaporate at room temperature. The reaction mixture was diluted with 20 ml of a THF/water 1 : 1 mixture, poured on ice and extracted with ethyl acetate (3 x 20 ml). The combined organic phases were dried over $MgSO_4$, filtered and concentrated under reduced pressure. Chromatography of the residue on silica gel with ethyl acetate (Rf = 0.29) yielding 172 mg (86%) of the diastereomeric alcohols **17** as colourless oil. As indicated by the integration ratio of the two signals of the 4-OH group, **17** occurs as 2 : 1 mixture of diastereomers.

^{1}H NMR (250 MHz, $CDCl_3$): δ = 0.85 (t, J = 6.4 Hz, 3 H, CH_3); 1.29 (t, J = 7 Hz, 6 H, $P(OCH_2CH_3)_2$); 1.21-1.49 (m, 19 H, $C(CH_3)_3$, Alkyl-CH_2); 1.54-1.92 (m, 4 H, PCH_2CH_2); 2.01 (m, 2 H, CH_2CH); 2.35 and 2.43 (d, br, 1 H, diastereomeric OH, integration ratio 2 : 1); 3.53 (m, 1 H, CHNH); 4.06 (dq, $^3J_{HH}$ = 7 Hz, $^3J_{HP}$ = 3.8 Hz, 4 H, $P(OCH_2CH_3)_2$); 4.15 (m, 1 H, CHOH); 4.76 (br, d, 1 H, NH); 5.44 (dt, J = 15.6 Hz, J = 6.1 Hz, 1 H, CH=CH); 5.78 (dt, J = 15.6 Hz, J = 6.2 Hz, 1 H, CH=CH).

^{13}C NMR (62.89 MHz, $CDCl_3$) values from the major diastereomer: δ = 13.74 (CH_3); 17.48, 17.67 ($P(OCH_2CH_3)_2$); 21.82, 23.29, 24.07, 24.36, 29.00, 29.25, 29.44, 29.54, 31.06, 31.14, 32.37 (Alkyl-CH_2, $C(CH_3)_3$, PCH_2CH_2); 56.40 (CHNH); 57.53 ($C(CH_3)_3$); 62.30, 63.41 ($P(OCH_2CH_3)_2$); 65.43 (CHOH); 132.16, 133.45 (C=C); 156.54 ($COOC(CH_3)_3$).

^{31}P NMR (121.49 MHz, $CDCl_3$): δ = 31.85.

Analysis: $C_{22}H_{44}NO_6P$ (449.291) calcd. (%): C 58.78, H 9.86, N 3.12; found (%): C 59.08, H 9.70, N 3.53; MS (FAB-MS): $C_{22}H_{45}NO_6P$ $[M+H]^+$, calcd.: m/z = 450.298, found: m/z = 450.30.

Diethyl-3-(S)-amino-4-hydroxy-5-tridecenyl-1-phosphonate hydrochloride (18)

The protected amino alcohols **17** (150 mg, 0.33 mmol) were dissolved in methanol saturated with hydrogen chloride (20 ml) and stirred for 12 h at ambient temperature. The reaction mixture was extracted once with some diethyl ether which was discarded. The solvent was evaporated under reduced pressure to afford 110 mg (85 %) of **18** as a yellow oil.

^{1}H NMR (250 MHz, D_2O): δ = 0.83 (t, J = 6.5 Hz, 3 H, CH_3); 1.27 (t, J = 7 Hz, 6 H, $P(OCH_2CH_3)_2$); 1.21-1.49 (m, 10 H, Alkyl-CH_2); 1.55-1.94 (m, 4 H, PCH_2CH_2); 2.04 (m, 2 H, CH_2CH); 3.56 (m, 1 H, $CHNH_3$); 4.04 (dq, $^3J_{HH}$ = 7 Hz, $^3J_{HP}$ = 3.7 Hz, 4 H, $P(OCH_2CH_3)_2$); 4.25 (m, 1 H, CHOH); 5.48 (dt, J = 15.4 Hz, J = 6.0 Hz, 1 H, CH=CH); 5.79 (dt, J = 15.4 Hz, J = 6.0 Hz, 1 H, CH=CH).

^{13}C NMR (62.89 MHz, D_2O) values from the major diastereomer: δ = 13.64 (CH_3); 17.58, 17.77 ($P(OCH_2CH_3)_2$); 21.72, 21.89, 23.49, 24.17, 24.26, 29.54, 31.16, 31.18 (Alkyl-CH_2, PCH_2CH_2); 57.40 ($CHNH_3$); 62.40, 63.41 ($P(OCH_2CH_3)_2$); 65.63 (CHOH); 132.56, 133.75 (C=C).

^{31}P NMR (121.49 MHz, D_2O): δ = 32.03.

Analysis: $ClC_{17}H_{37}NO_4P$ (385.218) calcd. (%): C 52.96, H 9.68, N 3.64, found (%): C 52.6, H 9.29, N 3.60; MS (FAB-MS): $C_{17}H_{37}NO_4P$ $[M+H^+-HCl]$, calcd.: m/z = 350.246, found: m/z = 350.30.

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