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Synthesis of Sphinganine Analogues modified in the Head Group

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Abstract: The synthesis of sphinganine analogues modified in the head group is reported. The target compounds are efficiently prepared by means of the Henry reaction. Synthesis and results of preliminary investigations of the derivatives as potential inhibitors of sphingolipid biosynthesis are presented.

Glycosphingolipids (GSL) are components of the plasma membrane of vertebrates. At the cell surface they form cell-type characteristic patterns which change with cell growth, differentiation, viral transformation and oncogenesis [1].

The biosynthesis of GSL [2] starts with the formation of ceramide (*N*-acylsphingenine) in several steps from L-serine at the cytosolic face of the endoplasmatic reticulum followed by step-wise glycosylation in the Golgi apparatus.

It is known that GSL interact with toxins, viruses, and bacteria as well as with membrane bound receptors and enzymes [3]. Recently, sialyl Lewis x determinants on GSL were found to be ligands of the endothelial leukocyte adhesion molecule [4]. Additionally, products of GSL catabolism, e. g. sphingenine and sphingenine-1-phosphate, are supposed to play a role in signal transduction events [5].

The biological functions of GSL are poorly understood since no genetic defects of eucaryotic GSL biosynthesis have been found and no specific inhibitors of GSL catabolism suitable for cell culture systems are available. L-Cycloserine [6], β-fluoro-L-alanine and β-chloro-L-alanine [7], and the sphingofungins [8] are reported as inhibitors of serine palmitoyl transferase, the enzyme catalyzing the committed step of GSL biosynthesis. Recently fumonisine B1, a mycotoxin of *Fusarium moniliforme*, has been found to inhibit dihydroceramide synthase [9]. Radin reported D-*threo*-1-phenyl-2-decanoylamino-3-morpholino-1-propanol to be an inhibitor of glucosyl ceramide synthase [10].

In this paper we describe the synthesis of structural analogues of sphinganine modified in the head group. Azidosphingenine (2*S*,3*R*,4*E*-2-azido-octadec-4-ene-1,3-diol), which is known as intermediate in the synthesis of D-*erythro*-sphingenine [11] and as glycosyl acceptor [12] in the synthesis of GSL [13], decreases the activity of serine palmitoyl transferase [14]. This observation prompted us to synthesize and investigate the effect of the corresponding sphinganine derivative **6**, in which the azido function is replaced by a nitro group. Furthermore, in several cases the nitro group is reported to behave like a carboxy function in biological systems [15] so that **6** shows some similarity with 2-carboxysphinganine, another head-group modified analogue designed and synthesized as potential inhibitor of GSL biosynthesis [16].

We decided to synthesize and investigate the 2- and 3-methyl derivatives **11a,b** and **12**, since the 4-methyl derivative [17] of *cis*-sphingenine inhibits GSL biosynthesis and alters the shape of neuronal cells treated with this compound [18].

The 2-hydroxymethyl derivative **16** (figure 2) was originally prepared as long chain analogue of tris-(hydroxymethyl)-methylamine, which is used as buffer substance and turned out to be a potent inhibitor of acid ceramidase [19], an enzyme of GSL catabolism. According to its structural similarity to **11a,b** and 2-carboxysphinganine (vide supra), it was also investigated towards its effect on GSL biosynthesis.

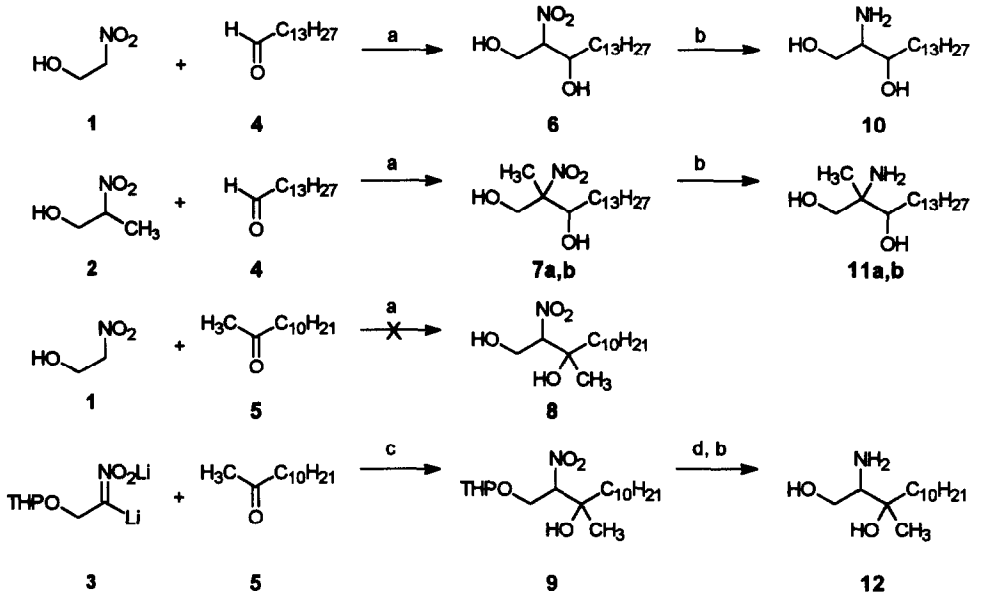

Figure 1: Approach to analogues **6**, **11a**, **11b** and **12** of sphinganine **10**, modified in the head group. a: cat. KOH, Et_2O, b: ammonium formate, Pd-C, MeOH, c: 1. THF, 2. HOAc, d: 1. HCl, 2. KOH. The diastereomers **7a** and **b** were separated and independently transformed to **11a** and **11b**. Other compounds are mixtures of diastereomers. Yields: **6** 64 %, **7a,b** 19%, **9** 38%, **10** 82 %, **11a** 86 %, **11b** 76 %, **12** 89 %.

The sphinganine analogues **6**, **11a,b**, **12** and **16** were efficiently prepared by means of the Henry reaction. The addition of aliphatic nitronates to aldehydes, the Henry reaction [20], is one of the classical methods for C-C bond formation. At the beginning of the fifties the addition of nitroethanol (**1**) to long chain aldehydes was used in the synthesis of sphinganine by Grob [21], at the end of the fifties in the synthesis of sphingenine in its four stereoisomeric forms [22]. ^3H- and ^{14}C-labelled sphingenine and sphinganine was prepared by Stoffel and Sticht according to this method in their corresponding stereoisomeric forms [23]. Recently, the method of Grob was utilized after modification by a Japanese group for the synthesis of sphinganine in various chain lengths [24], for the synthesis of racemic *erythro*-sphingenine [25] and for the synthesis of cerebrosides in their respective stereoisomeric forms [26]. Whereas the Henry reaction was successfully applied in the preparation of

sphingenine and sphinganine, it was hardly used in the synthesis of analogues of sphingolipids. (For the synthesis of some of these analogues see [27].) Possible reasons might be the moderate yields in which the target compounds are obtained or the fact that the products usually are mixtures of stereoisomers. The latter can be desirable for biochemical purposes since several stereoisomers could be assayed in one experiment. In the case reported by us the shortness of the reaction sequence in the synthesis of the target compounds compensates for the modest or even poor yields in the C-C bond forming step. In publications by Seebach et al. yields [28] and diastereoselectivity [29] in the synthesis of vicinal amino alcohols by this method could be improved. Additionally, when the preparative flexibility immanent to the nitro group [30] is considered, in spite of their disadvantages the Henry reaction bears a broad and insufficient utilized potential for the synthesis of sphingolipid analogue compounds.

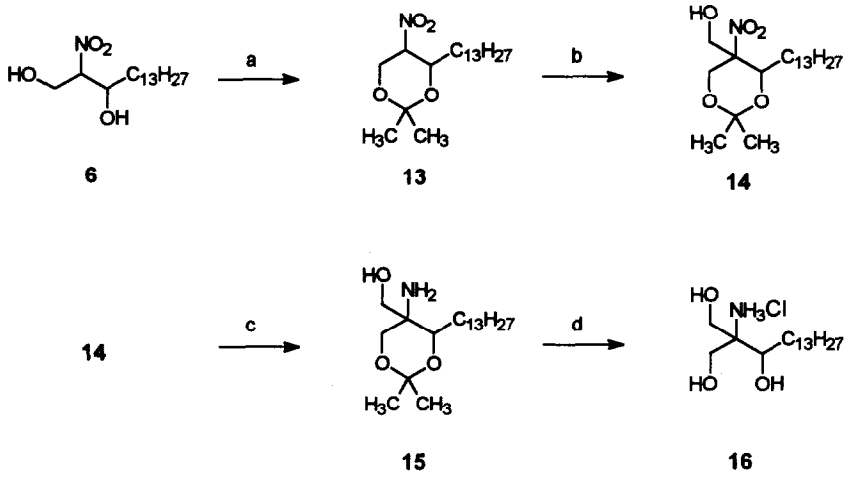

Figure 2: Approach to 2-hydroxymethyl-C_{16}-sphinganine (**16**). a: dimethoxypropane, acetone, *p*-toluenesulfonic acid, 61.5 %, b: formaldehyde, cat. KOH, Et_2O, 52.8 %, c: ammonium formate, Pd-C, MeOH, 59%, d: 1. trifluoroacetic acid, 2. HCl, 32.2 %.

Nitro-C_{16}-sphinganine (**6**) is readily available by addition of nitroethanol (**1**) to myristic aldehyde (**4**) (figure 1). Reduction of the intermediate nitro derivatives to the target compounds was achieved by transfer hydrogenation [31] as shown in the synthesis of racemic C_{16}-sphinganine (**10**) from **6**. Compared with **1**, the addition of the less reactive nitropropanol (**2**) to aldehyde **4** proceeds in only poor yield. Nevertheless the target compound **11** is available in only two steps by this method and should be less accessible on other ways. Additionally, the two diastereomers formed in the synthesis of **7** were readily separated into **7a** and **7b** and were independently transformed to the target compounds **11a** and **11b** by transfer hydrogenation.

The synthesis of **12** could not be achieved in this way, since the electrophile 2-dodecanone (**5**) turned out to be not reactive enough towards nitroethanol (**1**). No product **8** was detectable under the reaction conditions. Enhancement of the nucleophilicity of **1** by the method of Seebach et al. [32] was achieved by double lithiation of the protected derivative of **1**. The addition product of **3** and **5** was obtained in moderate yield and transformed to **12** by a one step procedure.

For the synthesis of the 2-hydroxymethyl derivative **16** (figure 2) nitrodiol **6** was protected as dioxane **13**. Under the reaction conditions the isomerization of the component with *threo* stereochemistry to the *erythro* derivative occurs, as reported for comparable derivatives [21, 26]. Addition of formaldehyde to the nitro compound **13** was achieved by the procedure used in the synthesis of the derivatives **6** and **7a,b**. Subsequent transfer hydrogenation and deprotection afforded the target compound **16** as the hydrochloride (figure 2).

The target compounds were investigated in primary cultured neurons from 6-7 day old mice according to ref. [14a]. Inhibition of sphingolipid biosynthesis was quantified as reduction of L-[3-^{14}C]serine incorporation into sphingolipids. Preliminary results are summarized in table 1:

compound	inhibition of [^{14}C]-serine incorporation into sphingolipids
6	10 µM: 30 %
11a	25 µM: 69 %
11b	25 µM: no inhibition
12	10 µM: no inhibition
16	10 µM: no inhibition

Table 1: preliminary results achieved with the synthesized compounds

With the exception of **6** and **11a** the modification of the sphinganine head group described here leads to compounds which are no inhibitors of sphingolipid biosynthesis according to preliminary studies. **6** and **11a** inhibit incorporation of [^{14}C]-serine into sphingolipids. Inhibition studies with **11a** *in vitro* [14b] suggest that the inhibition proceeds on the level of sphinganine-*N*-acyltransferase, whereas serine palmitoyl transferase is not inhibited in 1 mM concentration. The inhibition seems to be specific since the diastereomeric compound **11b** shows no inhibition under the same conditions in cell culture. To date the relative configuration of the diastereomers is not known. Compared with the known inhibitor of sphinganine-*N*-acyltransferase fumonisine (vide supra, IC_{50} = 0.1 µM in rat liver microsomes), **11a** is a weaker inhibitor for about two orders of magnitude. Possible improvements of the lead compound **11a** might be the variation of chain length, the generation of the enantiomeric pure compound by resolution of the racemic mixture or the introduction of conformational rigidity.

Experimental

Solvents were purified in the usual way; melting points are uncorrected. Optical rotations: Perkin-Elmer polarimeter P 241; thin layer chromatography: glass plates Kieselgel 60 (Merck, layer thickness 0.2 mm); column chromatography: Kieselgel 60 (Merck, 0.063 - 0.200 mm). ^{1}H-NMR: Bruker WH 90, Bruker AC-200. Mass spectrometry: 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.

General procedure 1: nitroaldol addition

To the nitro component (1 equivalent, 10 mmol in diethyl ether) a solution of potassium hydroxide (1/35 equivalent, 15 % in methanol) is added and the mixture cooled to 0°C. The aldehyde component (1 equivalent,

10 mmol in 10 ml diethyl ether is slowly added with stirring. After complete addition the cooling bath is removed and stirring is continued at room temperature until satisfactory turnover is indicated by thin layer chromatography (tlc). After addition of acetic acid (0.25 ml for 10 mmol educt, excess) the solvent is removed under reduced pressure and the remaining residue is purified by column chromatography using silica gel and *n*-hexane - ethyl acetate as the eluent.

General procedure 2: transfer hydrogenation

The nitro compound is dissolved in methanol, palladium on charcoal (10%; caution: pyrophoric) and ammonium formate (10 equivalents, excess) is added. The suspension is stirred and saturated with nitrogen. If necessary as indicated by tlc, additional ammonium formate is added. After stirring for 16 hours at room temperature the catalyst is filtered off, methanol is removed under reduced pressure and the residue is taken up in 1 N hydrochloric acid. The aqueous phase is extracted with dichloromethane several times, made alkaline with solid potassium hydroxide and extracted three times with dichloromethane. The organic layer is dried over magnesium sulphate and evaporated. The remaining residue is usually homogenous according to tlc.

2-Nitro-hexadecan-1,3-diol (6)

2-Nitro-hexadecan-1,3-diol **(6)** is synthesized according to general procedure 1. Nitroethanol **(1)** (14 torr, 114°C) and myristic aldehyde **(2)** (0.1 torr, 125°C) were distilled before use. Preparation: 17.6 mmol, yield: 3.42 g (64.0 %) **6** as a solidifying oil, R_f (*n*-hexane: ethyl acetate = 2 : 1) = 0.53 and 0.37.
^{1}H-NMR (200 MHz, $CDCl_3$): δ = 0.80 - 0.95 (m, 3H; CH_3), 1.10 - 1.65 (m; 24 H; CH_2), 2.15 - 2.70 (m, br., 2 H; OH), 4.00 - 4.35 (m, 3H; CH_2O, CHO), 4.40 - 4.49 (m, 0.5 H; $CHNO_2$), 4.53 - 4.63 (m, 0.5H; $CHNO_2$).
Analysis: $C_{16}H_{33}NO_4$ (303.44) calcd. (%): C 63.33, H 10.96, N 4.62; found (%): C 63.50, H 10.98, N 4.81; MS (FAB-MS): $C_{16}H_{34}NO_4$ $[M+H]^+$, *m/z* = 304.3.

2-Methyl-2-nitro-hexadecan-1,3-diol (7a,b)

7a,b was synthesized according to general procedure 1 from 2-nitropropanol **(2)** and freshly distilled myristic aldehyde **(4)**. Preparation: 7.7 mmol, reaction time: 24 hours, yield: 462 mg (18.9 %) of a colourless oil. Chromatography on silica gel afforded two fractions corresponding to the two diastereomers. The fractions were isolated in a ratio of 49.5 : 50.5.
7a: R_f (*n*-hexane : ethyl acetate = 3 : 1) = 0.30. ^{1}H-NMR (200 MHz, $CDCl_3$): δ = 0.88 (t, J = 8 Hz, 3H; 16-CH_3), 1.10 - 1.65 (m; 27 H, with a s at 1.53 ppm; 12 x CH_2 and 2-CH_3), 2.25 - 2.45 (m, 2 H; OH), 3.90 - 4.15 (m, 3H; CH_2O, CHO).
Analysis: $C_{17}H_{35}NO_4$ (317.47) calcd. (%): C 64.32, H 11.11, N 4.41; found (%): C 65.07, H 11.43, N 4.24.

7b: R_f (*n*-hexane : ethyl acetate = 3 : 1) = 0.21. ^{1}H-NMR (200 MHz, $CDCl_3$): δ = 0.88 (t, J = 8 Hz, 3H; 16-CH_3), 1.10 - 170 (m; 27 H, with a s at 1.45 ppm; 12 x CH_2 and 2-CH_3), 2.57 (d, J = 7 Hz, 1H; 3-O<u>H</u>), 2.74 (t, J = 7 Hz, 1H; 1-OH), 4.04 (d, J = 7 Hz, 2H; 1-CH_2), 4.18 (q, J = 7 Hz, 1H; 3-H).
Analysis: $C_{17}H_{35}NO_4$ (317.47) calcd. (%): C 64.32, H 11.11, N 4.41; found (%): C 65.20, H 10.02, N n.d.; MS (70 eV): $C_{16}H_{32}NO_3$ $[M-CH_2OH]^+$, calcd.: *m/z* = 282.2433, found: *m/z* = 282.2460; $C_{17}H_{36}NO_2$ $[M-NO_2+H]^+$, calcd.: *m/z* = 272.2715, found: *m/z* = 272.2696.

3-Methyl-2-nitro-tridecan-1,3-diol (8)

Conversion of nitroethanol **(1)** and 2-dodecanone **(5)**, both components freshly distilled, according to general procedure 1, was not detectable. The synthesis of the target compound **12** was achieved by reaction of the double lithiated THP-ether of **1** with **5** via the intermediate **9**.

3-Methyl-2-nitro-1-(2-tetrahydropyranyloxy)-tridecan-3-ol (9)

2-Nitroethyl-(2-tetrahydropyranyl)-ether (1ml, 1.16 g, 6.62 mmol) is dissolved in dry THF (10 ml) and cooled to -78°C under argon atmosphere. *n*-Butyllithium (8.4 ml of a 1.6 M solution in *n*-hexane, 13.6 mmol) is added dropwise at -78°C and stirring is continued for two hours at this temperature. A solution of 2-dodecanone **(5)** (1.22 g, 6.62 mmol) in dry THF (5 ml) is added dropwise. After stirring for three hours at -78°C acetic acid (0.94 ml, 16.5 mmol) is added and the reaction mixture was brought to room temperature. Volatile components were distilled off and the remaining residue was chromatographed on silica gel with *n*-hexane : ethyl acetate = 3 : 1 as the eluent. **9** was obtained as a colourless oil, R_f = 0.47, yield = 919 mg (38.6%).

^{1}H-NMR (90 MHz, $CDCl_3$): δ = 0.86 (t, J = 6 Hz, 3H; 13-CH_3), 0.88 - 1.89 (m; 27 H; 12 x CH_2, 3-CH_3), 2.33 - 2.55 (m, 1 H; OH), 3.33 - 4.89 (m, 6H; 1-CH_2, 2-H, 2'-H, 6'-CH_2).
Analysis: $C_{19}H_{37}NO_5$ (359.51) calcd. (%): C 63.48, H 10.37, N 3.90; found (%): C 63.10, H 10.29, N 4.02; MS (FAB-MS): $C_{19}H_{38}NO_5$ $[M+H]^+$, *m/z* = 360.3.

2-Amino-hexadecan-1,3-diol (**10**)
Sphinganine **10** was synthesized according to general procedure 2. Preparation: 614 mg (2.02 mmol) **6**; yield: 456 mg (1.67 mmol, 82.6 %) **10** as a waxy solid, R_f (chloroform : methanol : 2 N ammonia = 40 : 10: 1) = 0.38.
^{1}H-NMR (200 MHz, $CDCl_3$): δ = 0.87 (t, J = 6 Hz, 3 H; CH_3), 1.15 - 1.55 (m, 24 H; CH_2), 2.10 - 2.30 (m, br., 4 H; 2 OH, NH_2), 2.70 - 2.90 (m, 1 H; 2-H), 3.45 - 3.80 (m, 3 H; 1-CH_2, 3-H).
Analysis: $C_{16}H_{35}NO_2$ x 1.5 H_2O (300.48) calcd. (%): C 63.95, H 12.75, N 4.66; found (%): C 64.07, H 12.90, N 4.12; MS (70 eV): $C_{16}H_{36}NO_2$ $[M+H]^+$, calcd.: *m/z* = 274.2746, found: *m/z* = 274.2739.

2-Amino-2-methyl-hexadecan-1,3-diol (**11a,b**)
11a was obtained from **7a** according to general procedure 2; **11b** accordingly from **7b**.

11a: Preparation: 214 mg (0.67 mmol) **7a**, yield: 167 mg (86.1%) **11a**, R_f (chloroform : methanol : 2 N ammonia = 40 : 10 : 1) = 0.44.
^{1}H-NMR (90 MHz, $CDCl_3$): δ = 0.60 - 1.09 (m, 6 H; 2 x CH_3), 1.10 - 1.78 (m, 24 H; CH_2), 2.22 (m, br., 4 H; 2 OH, NH_2), 3.10 - 3.80 (m, 3 H; 1-CH_2, 3-H).
MS (70 eV): $C_{17}H_{38}NO_2$ $[M+H]^+$, calcd.: *m/z* = 288.2902, found: *m/z* = 288.2899.

11b: Preparation: 186 mg (058 mmol) **7b**, yield: 128 mg (75.9 %) **11b**, R_f (chloroform : methanol : 2 N ammonia = 40 : 10 : 1) = 0.52.
^{1}H-NMR (200 MHz, $CDCl_3$): δ = 0.87 (t, J = 6.5 Hz, 3 H; 16-CH_3), 1.00 (s, 3H; 2-CH_3) 1.15 - 1.45 (m, 22 H; CH_2), 1.46 - 1.64 (m, 2 H; 4-CH_2), 2.28 (m, br., 4 H; NH_2, 2 x OH), 3.25 - 3.40 (m, 2H; 1-CH_2), 3.65 - 3.78 (m; 1H, 3-H).
Analysis: $C_{17}H_{37}NO_2$ (287.49) calcd. (%): C 71.03, H 12.97, N 4.87 found (%): C 70.47, H 12.41, N n.d.; MS (70 eV): $C_{17}H_{38}NO_2$ $[M+H]^+$, calcd.: *m/z* = 288.2903, found: *m/z* = 288.2872.

2-Amino-3-methyl-tridecan-1,3-diol (**12**)
12 was prepared from **9** according to general procedure 2 with the exception, that stirring in HCl was continued until complete cleavage of the THP-ether before extraction with dichloromethane. Preparation: 780 mg (2.17 mmol) **9**, yield: 474 mg (1.93 mmol, 88.9 %) as a colourless oil. As indicated by the integration ratio of the two signals of the 3-Methyl group, **12** occurs as 1:1-mixture of diastereomers.
^{1}H-NMR (200 MHz, $CDCl_3$): δ = 0.87 (t, J = 6.5 Hz, 3 H; 13-CH_3), 1.10 - 1.70 (m, 23 H with 2 s at 1.12 and 1.17 ppm; diastereomeric 3-CH_3, 9 x CH_2, 2 x OH), 2.48 (m, br., 2 H; NH_2), 2.72 (m, 1 H; 2-H), 3.50 - 3.80 (m, 2 H; 1-CH_2).
Analysis: $C_{14}H_{31}NO_2$ x 0.5 H_2O (254.41) calcd. (%): C 66.09, H 12.68, N 5.50; found (%): C 66.23, H 12.23, N5.28; MS (70 eV): $C_{14}H_{32}NO_2$ $[M+H]^+$, calcd.: *m/z* = 246,2433, found: *m/z* = 246,2421.

erythro-2,2-Dimethyl-5-nitro-4-tridecanyl-1,3-dioxane (**13**)
Nitrodiol **6** (1.16 g, 3.82 mmol), 2,2-dimethoxy propane (18.7 ml, 153 mmol), acetone (6 ml) and *p*-toluene sulfonic acid monohydrate (73 mg, 0.38 mmol) are refluxed until complete conversion is indicated by tlc. After the mixture has cooled down to room temperature it is diluted with ethyl acetate (100 ml) and extracted with brine. The organic layer is dried over magnesium sulphate and concentrated under reduced pressure. The remaining oil is chromatographed on silica gel with *n*-hexane : ethyl acetate = 10 : 1 (R_f = 0.51) as the eluent. A colourless, slowly solidifying oil is obtained in 808.7 mg (61.5 %) yield.
^{1}H-NMR (200 MHz, $CDCl_3$): δ = 0.87 (t, J = 6.5 Hz, 3 H; 13'-CH_3), 1.24 (m, 22 H; CH_2), 1.38 (s, 3 H; CH_3), 1.49 (s, 3 H; CH_3), 1.56 (m, 2 H; 1'-CH_2), 4.10 (dd, J = 12 Hz, J = 5.6 Hz, 1 H; 6-H_A); 4.16 - 4.24 (m, 1 H; 4-H), 4.22 (dd, J = 12 Hz, J = 7 Hz, 1 H; 6-H_B), 4.43 (ddd, J = 9 Hz, J = 7 Hz, J = 5.6 Hz, 1 H; 5-H).
Analysis: $C_{19}H_{37}NO_4$ (343.51) calcd. (%): C 66.44, H 10.86, N 4.08; found (%): C 65.72, H 11.08, N 3.90; MS (70 eV): $C_{19}H_{38}NO_4$ $[M+H]^+$, calcd.: *m/z* = 344.2801, found: *m/z* = 344.2847.

2,2-Dimethyl-5-hydroxymethyl-5-nitro-4-tridecanyl-1,3-dioxane (14)

The synthesis of compound **14** proceeded according to general procedure 1 with the exception that formaldehyde is introduced at room temperature as a gas into the reaction mixture. Preparation: 500 mg (1.46 mmol) **13** in 25 ml diethyl ether. Formaldehyde was generated by thermal decomposition of paraformaldehyde (1 g, excess) at 160 - 180°C. Yield: 288 mg (52.8 %) of a colourless oil, R_f (*n*-hexane: ethyl acetate = 8 : 1) = 0.16.

^{1}H-NMR (90 MHz, $CDCl_3$): δ = 0.82 (t, J = 6 Hz, 3 H; CH_3), 1.00 - 1.58 (m, 30 H with two s at 1.37 and 1.46 ppm; 12 x CH_2 and 2 CH_3), 1.89 (m, br., 1 H; OH), 3.97 - 4.44 (m, 5 H; 6-CH_2, 1"-CH_2, 4-H).

Analysis: $C_{20}H_{39}NO_5$ (373.54) calcd. (%): C 64.31, H 10.52, N 3.75; found (%): C 64.32, H 10.48, N 3.88; MS (FAB-MS): $C_{20}H_{40}NO_5$ $[M+H]^+$, *m/z* = 374.2.

5-Amino-2,2-dimethyl-5-hydroxymethyl-4-tridecanyl-1,3-dioxane (15)

15 was obtained from **14** according to general procedure 2 in 59 % yield.

^{1}H-NMR (90 MHz, $CDCl_3$): δ = 0.81 (t, J = 5 Hz, 3 H; CH_3), 1.00 - 1.78 (m, 33 H; 12 x CH_2, 2 x CH_3, NH_2, OH), 3.23 - 4.05 (m, 5 H; 2 x CH_2O, CHO).

Analysis: $C_{20}H_{41}NO_3$ (343.55) calcd. (%): C 69.92, H 12.03, N 4.08; found (%): C 69.37, H 12.11, N 3.98; MS (70 eV): $C_{20}H_{41}NO_3$ $[M]^+$, calcd.: *m/z* = 343.3086, found: *m/z* = 343.3073.

2-Amino-2-hydroxymethyl-hexadecan-1,3-diol hydrochloride (16)

15 (107 mg, 0.31 mmol) is dissolved in trifluoroacetic acid (10 ml) and stirred for 48 hours at room temperature. Trifluoroacetic acid is removed under reduced pressure and the residue is evaporated twice with 5 % hydrochloric acid. A white, waxy solid (35 mg, 0.10 mmol, 32.2 %) is precipitated with diethyl ether from ethanol.

^{1}H-NMR (200 MHz, [D_6]DMSO): δ = 0.87 (t, J = 6 Hz, 3 H; CH_3), 1.05 - 1.65 (m, 24 H; CH_2), 3.40 - 3.70 (m, 5 H; 2 x CH_2OH, CHOH), 5.05 - 5.35 (m, 3H, OH), 7.45 - 7.80 (m, 3H; NH_3^+).

Analysis: $C_{17}H_{38}NO_3Cl$ x 0.25 H_2O (344.45) calcd. (%): C 59.28, H 11.25, N 4.07; found (%): C 58.91, H 10.98, N 4.02; MS (FAB-MS): $C_{17}H_{38}NO_3$ $[M+H-HCl]^+$, *m/z* = 304.2.

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