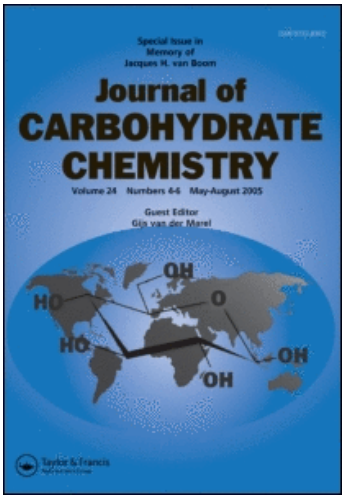

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SYNTHESIS OF 2-CARBOXY-SUBSTITUTED SPHINGOSINE DERIVATIVES[1]

Narrinder P. Singh[a], Athanassios Giannis[b], Elfi Henk[b], Thomas Kolter[b], Konrad Sandhoff[b], and Richard R. Schmidt*[a]

[a]Fakultät für Chemie, Universität Konstanz Postfach 5560, D-7750 Konstanz, Germany

[b]Institut für Organische Chemie und Biochemie, Universität Bonn, Gerhard-Domagk-Str. 1, D-5300 Bonn, Germany

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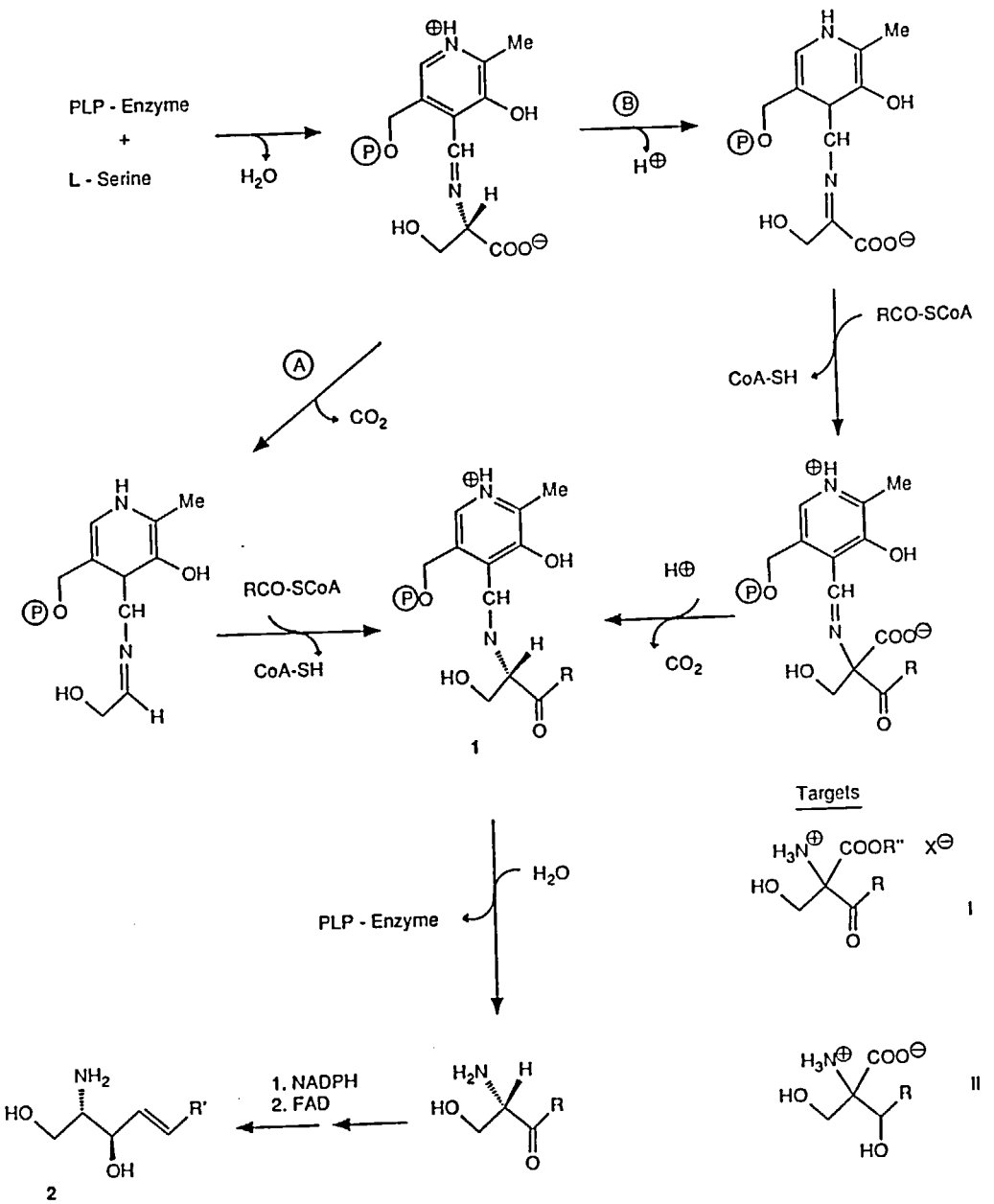
ABSTRACT

From the sphingosine biosynthesis pathway it is deduced that 2-carboxy-substituted sphinganine derivatives should be suitable inhibitors of sphingosine biosynthesis. For their synthesis enantioselective acylation and α-hydroxyalkylation of serine was performed via its optically pure 2-*tert*-butyl-oxazolidine derivatives **4A,B** and ent-**4A**, known to undergo partial chirality transfer from serine to the 2-position of **4** and then to the 4-position. Thus, after acid hydrolysis compounds *R*-**7Aa**, -**7Ab**, -**7Ac**, -**7Bb**, *S*-**7Aa**, -**7Ab**, and **11** are provided highly stereoselectively.

INTRODUCTION

The importance of glycosphingolipids in cellular regulation has recently created a growing interest in the chemistry and biology of sphingosines.[2-7] Moreover, lysosphingolipids and sphingosine itself exhibit protein kinase C



Scheme 1

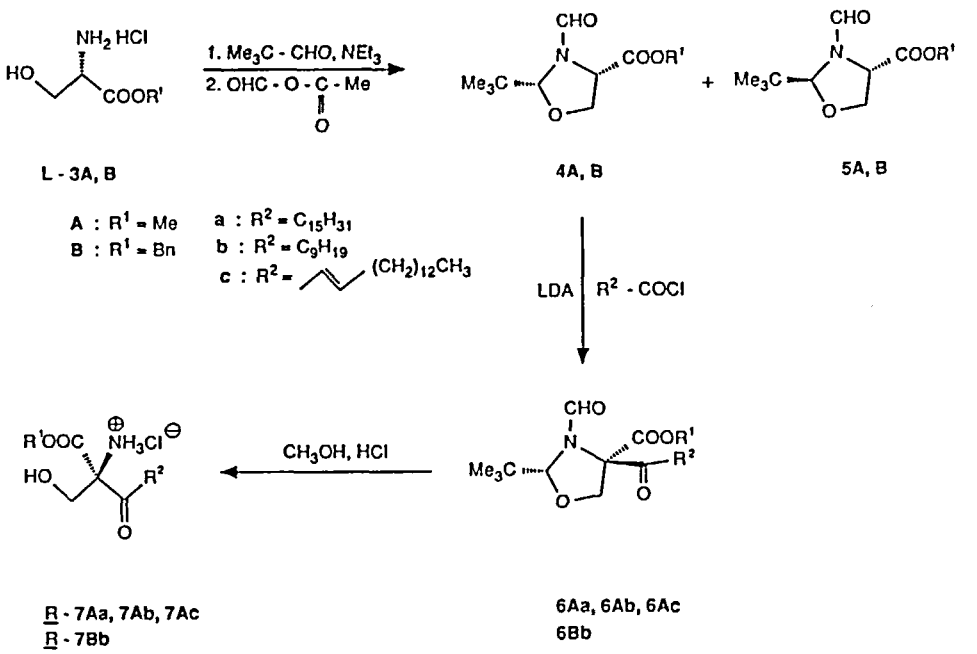
inhibitor activity and other biological effects, thus, stimulating additional scientific efforts to unravel their biological functions.[5] Therefore, the biosynthesis of sphingosines (Scheme 1)[6,8-11] and inhibition of the biosynthesis became of prime importance.[4,5,12]

The metabolic pathway starts from **L**-serine and a pyridoxal-phosphate-enzyme (PLP-enzyme) which reacts with palmitoyl-SCoA to provide, via a **D**-2-amino-1-hydroxy octadecanone-3 intermediate **1**, the **D**-*erythro*-C_{18}-sphingosine **2**. Two pathways, (A) and (B) are conceivable differing in decarboxylation (A) or deprotonation (B), respectively, as first steps in the synthesis of the intermediate **1**. Experimental evidence is in support of pathway (B).[10] Subsequent NADPH-dependent carbonyl group reduction and FAD-dependent dehydrogenation (possibly after ceramide formation) conclude the biosynthesis.

Obviously, 2-carboxy-substituted sphingosine derivatives of type I or II could exhibit strong binding to the PLP-enzyme due to their structural similarity to the reaction intermediates; however, decarboxylation of the enzyme complex will be prevented due to the ester moiety (type I) or due to the 3-hydroxy group (type II), respectively. Investigations towards the enantioselective synthesis of these possible enzyme inhibitors will be reported in this paper.[13]

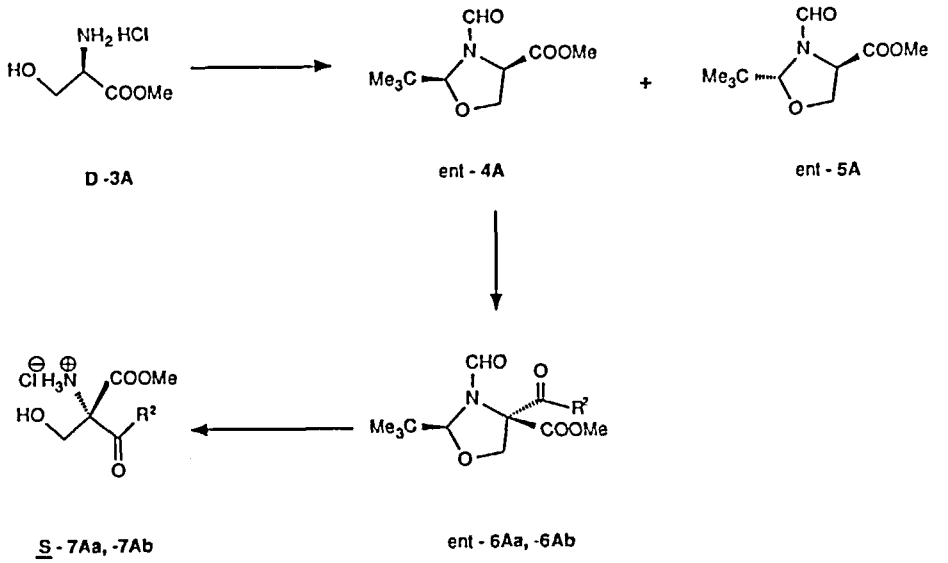
RESULTS AND DISCUSSION

Enantioselective acylation or α-hydroxyalkylation in 2-position of **L**- or **D**-serine, respectively, would provide a most convenient route for the synthesis of these compounds. However, the required 2-deprotonation of simple *O,N*-protected serine derivatives would result in complete racemisation. Seebach and Aebi developed a method where partial chirality transfer from C-2 of serine esters **3** to the C-2 of oxazolidine derivatives **4** (Scheme 2) and back to C-2 substituted serine derivatives (for instance, via **6** and **7**) is possible.[14,15]



Scheme 2

The required starting materials **4A,B** were obtained from serine esters **L-3A,B** as described[14,16] (**4A**) or according to the literature procedure (**4B**), respectively. Small amounts of the diastereoisomers **5A,B** were separated by crystallisation of the crude product. Treatment of oxazolidine derivative **4A** with lithium diisopropylamide (LDA) at -78 °C and then with palmitoyl chloride, finally at -100 °C, afforded practically exclusively the C-4 acylated compound **6Aa** in good yield. The structure is assigned in accord with reported findings;[14,15] thus, acylation takes place highly stereoselectively from the site opposite to the *tert*-butyl group. Similarly, compounds **6Ab** and **6Bb** were obtained from **4A,B** and decanoyl chloride and compound **6Ac** from **4A** and 2*E*-2-hexadecenoyl chloride,[17] respectively. Acid catalyzed removal of the *N*-formyl and the pivaldehyde protective groups from compounds **6** turned out to be a very critical step because side reactions were observed. Finally, slow addition

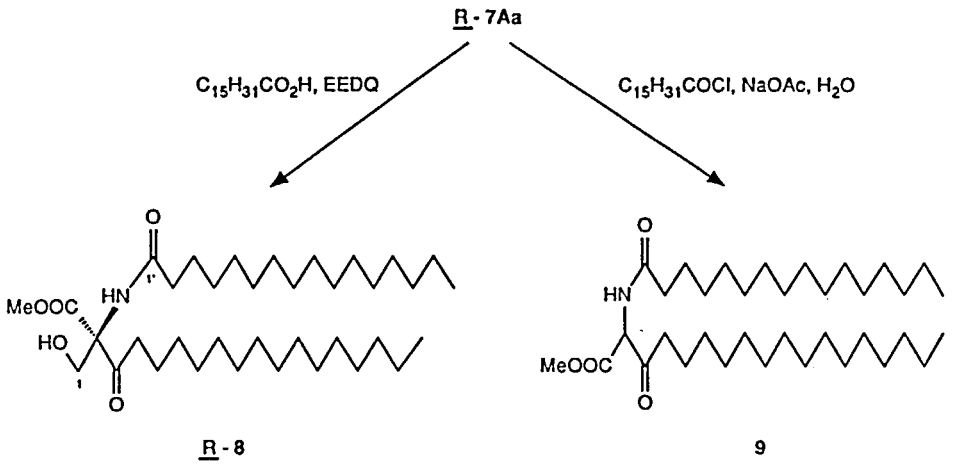


Scheme 3

of concentrated hydrochloric acid to methanolic solutions of compounds **6** furnished the desired products *R*-**7Aa**, **-7Ab**, **-7Ac**, and **-7Bb**, respectively, in good yields.

The configuration of the 2-carboxylated intermediate of the sphingosine biosynthesis is unknown; therefore, the *S*-enantiomers are also required for biological testing. Analogous formation from diastereomer **5A**, present in small amounts in the crude of **4A**, was not feasible. Therefore, **D**-serine methylester **D-3A** was utilized for the same reaction course (Scheme 3). Via enantiomers ent-**4A** and then ent-**6Aa**, **-6Ab** the *S*-enantiomers *S*-**7Aa** and *S*-**7Ab** were obtained in practically identical yields and diastereoselectivities.

N-Palmitoylation of compound *R*-**7Aa** with palmitic acid and EEDQ as condensing agent provided cleanly the corresponding ceramide derivative *R*-**8** (Scheme 4). However, when this reaction was carried out with palmitoyl chloride in presence of sodium acetate in water, concomitant retro-aldol reaction occurred, affording the 2-acylamino-3-oxo-octadecanoate **9** as a racemate.

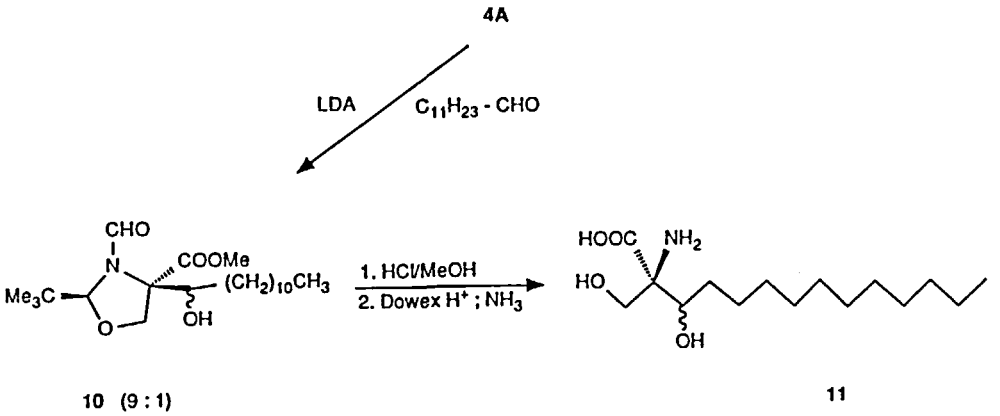


Scheme 4

α-Hydroxyalkylation of compound **4A** (Scheme 5) was performed by treatment with LDA at -78 °C and then by addition of laurinaldehyde providing a 9:1-mixture of two diastereomers **10** in only modest yield; the stereochemistry at the new hydroxy group is undefined. Deprotection of these compounds in 6N HCl in methanol under reflux afforded the desired α-carboxy substituted sphinganine derivatives **11**.

EXPERIMENTAL

General Procedures. Melting points are uncorrected. Analytical thin layer chromatography was performed on Kieselgel plates (0.25 mm thickness) obtained from E. Merck AG, Darmstadt (BRD). Preparative chromatography was performed on Kieselgel 60 (0.062-0.20 mm), obtained from Merck. Medium pressure liquid chromatography was performed on Kieselgel "LiChroprep" Si 60, 15-25 μm, using a refractive index detector. In all cases small samples were either finally purified through medium pressure liquid chromatography or through crystallisation. Specific rotations were determined with a PERKIN-ELMER 241 MC polarimeter. ^1H NMR spectra were recorded with a "JEOL-GX 400" (400 MHz) and a



10 (9 : 1) 11

Scheme 5

BRUKER "AC 250 Cryospec" (250 MHz) spectrometers, using TMS as internal standard. While citing ^1H NMR data the following abbreviations have been used: s (singlet), d (doublet), t (triplet), q (quartet), dd (double doublet), dt (double triplet) and br s (broad singlet). Peak positions have been assigned either on the basis of coupling constants or from the reported peak positions of relevant structures.

L-Serine methyl ester hydrochloride L-3A, L-Serine benzyl ester Hydrochloride L-3B, and **D-Serine methyl ester hydrochloride D-3A** were prepared according to the general procedures reported in the literature.[14,16]

Methyl (2R,4S)-2-Tert-butyl-3-formyl-oxazolidine-4-carboxylate (4A). Compound **4A** was accomplished by the reported procedure[14] and its spectral data were in accord to the reported.

Benzyl (2R,4S)-2-Tert-butyl-3-formyl-oxazolidine-4-carboxylate (4B). To 60 g (260 mmol) of finely powdered **L**-serine benzyl ester hydrochloride (**3B**) in 500 mL pentane was added 41.8 mL (300 mmol) of triethylamine and 56.5 mL (520 mmol) of 2,2-dimethylpropionaldehyde dropwise, under vigorous stirring. The mixture was heated at reflux for 16 h.

The water formed was azeotropically removed using a Dean-Stark trap. After 16 h no more water was separated. Reaction mixture was cooled to room temperature and filtered. The $Et_3N \cdot HCl$ thus formed was removed through filtration. The residue was washed with diethyl ether (3 x 150 mL) and filtered. The organic layers were combined, and the solvents removed in a rotatory evaporator. The residue thus obtained was dried for 24 h (room temperature) under high vacuum and gave 54.5 g (79.8%) of the reaction product. To 50 g of this residual product in anhydrous diethyl ether (250 mL) was added 70 mL of formic acetic anhydride at 0 °C, dropwise, with stirring. The reaction mixture was stirred at this temperature for 15 h and added $NaHCO_3$-solution (100 mL satd. $NaHCO_3$ in 60 g ice). The total mixture was taken in a 2 L separating funnel and 500 mL of diethyl ether was added. The organic phase was washed with water 4 x 100 mL, satd. $NaHCO_3$-solution (carefully, with slow additions) 4 x 70 mL, and satd. NaCl (100 mL). The organic phase was dried ($MgSO_4$) and the solvent removed in a rotatory evaporator under reduced pressure and the residue treated with ether/pentane for crystallisation. This afforded 44.0 g (79.5%; 63.5% overall yield) of fine colourless needle type crystals, having an R_F 0.66 in petroleum ether/ethyl acetate (1:1); mp 98-99 °C; $[\alpha]_D^{20} = -36.13°$ (*c* = 3.8, $CHCl_3$). ^1H NMR ($CDCl_3$, 250 MHz): 8.47, 8.39 (2s, 1H, formyl proton), 7.35 (m, 5H, aromatic protons), 5.19 (m, 2H, benzylic protons), 4.94 (dd, J = 3.0 Hz, 1H, H-C(4)), 4.87 (s, 1H, H-C(2)), 4.55 (m), 4.45 (dd, J = 3.0 Hz, 1H, H-C(5)), 4.06 (dd, J = 8.0 Hz, 1H, H-C(5)), 4.06 (dd, J = 8.0 Hz, 1H, H-C(5)), 0.92, 0.87 (2s, 9H, 3 x CH_3).

Anal. Calcd for $C_{16}H_{21}NO_4$: C, 65.96; H, 7.26; N, 4.80. Found: C, 65.99; H, 7.32; N, 4.88.

Methyl (2R,4R)-2-Tert-butyl-3-formyl-4-hexadecanoyl-oxazolidine-4-carboxylate (6Aa).- To a freshly prepared solution of LDA (6.48 mmol) in THF/hexane (10:1) 33 mL at -100 °C was added a solution of compound **4A** 1.27 g (5.89 mmol) in THF (6 mL) over 5 min. The reaction mixture was

stirred for 15 min at -78 °C. Then a solution of palmitoyl chloride 1.94 g (7.07 mmol) in THF (6 mL) was added dropwise over a period of 15 min. The reaction mixture was stirred for 3 h at -100 °C and quenched with a satd solution of ammonium chloride (50 mL), and the mixture was diluted with diethyl ether (500 mL). The organic layer was extracted and washed with water (100 mL) and dried ($MgSO_4$). The solvents were removed in a rotatory evaporator under reduced pressure to give a residual oil, which was chromatographed on a column of silica gel using petroleum ether/ethyl acetate (9:1) as eluents to afford 1.60 g (60%) as a colourless viscous oil, having R_F 0.52 in petroleum ether/ ethyl acetate (4:1); $[\alpha]_D^{20}$ = -3.02° (*c* = 2.58, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): 8.33, 8.25 (2s, 1H, formyl proton), 5.48, 5.08 (2s, 1H, H-C(2)), 4.66 (dd, J = 10.2, 9.0 Hz, 1H, H-C(5)), 4.13 (dd, J = 10.2, 9.0 Hz, 1H, H-C(5)), 3.88, 3.85 (2s, 3H, OCH_3), 2.68 (dt), 2.51 (dt, J = 10.2, 7.6 Hz, 2H, 2H-C(2')), 1.61 (m, 2H, 2H-C(3')), 1.26 (m, 24H, 12 x CH_2 aliphatics), 0.98, 0.94 (2s, 9H, 3 x CH_3), 0.88 (t, J = 6.3 Hz, 3H, CH_3).

Anal. Calcd for $C_{26}H_{47}NO_5$: C, 68.83; H, 10.44; N, 3.08. Found: C, 68.92; H, 10.65; N, 3.08.

Methyl (2R,4R)-2-Tert-butyl-3-formyl-4-decanoyl-oxazolidine-4-carboxylate (6Ab). Compound **6Ab** was prepared in a manner similar to **6Aa** as given above in a yield of 88%, as a viscous colourless oil, having an R_F 0.47 in petroleum ether/ethyl acetate (4:1); $[\alpha]_D^{20}$ = -3.42° (*c* = 1.87, $CHCl_3$); ^1H NMR ($CDCl_3$, 250 MHz): 8.25, 8.245 (2s, 1H, formyl proton), 5.48, 5.08 (2s, 1H, H-C(2)), 4.67 (dd, J = 10.2, 9.1 Hz, 1H, H-C(5)), 4.13 (dd, J = 10.2, 9.1 Hz, 1H, H-C(5)), 3.88, 3.85 (2s, 3H, OCH_3), 2.55 (m, 2H, 2H-C(2')), 1.60 (m, 2H, 2H-C(3')), 1.26 (m, 12H, 6 x CH_2 aliphatics), 0.98, 0.94 (2s, 9H, 3 x CH_3), 0.88 (t, J = 6.3 Hz, 3H, CH_3).

Anal. Calcd for $C_{20}H_{35}NO_5$: C, 65.01; H, 9.54; N, 3.79. Found: C, 65.07; H, 9.53; N, 4.0.

Methyl (2R,4R)-2-(Tert-butyl)-3-formyl-4-[(2E)-2-hexadecenoyl]-oxazolidine-4-carboxylate (6Ac). Compound **6Ac** was prepared according to the above given procedure for compound **6Aa**. The required 2*E*-2-hexadecenoyl chloride was prepared according to the reported[17] procedure. Compound **6Ac** was obtained in a yield of 20%, having R_F 0.53 in petroleum ether/ethyl acetate (4:1); $[\alpha]_D^{20}$ = -3.21° (*c* = 1.12, $CHCl_3$); ^1H NMR ($CDCl_3$, 250 MHz): 8.34, 8.20 (2s, 1H, formyl proton), 7.10 (m, 1H, H-C(3')), 6.22 (m, 1H, H-C(2')), 5.5 (s), 5.39 (m), 5.10 (s, 1H, H-C(2)), 4.67 (dd, J = 10.3, 9.4 Hz, H-C(5)), 4.20 (dd, J = 10.3, 9.4 Hz, H-C(5)), 3.85, 3.84, 3.82, 3.78 (4s, 3H, OCH_3), 2.20 (m, 2H, 2H-C(4')), 1.40 (m, 2H, 2H-C(5')), 1.23 (m, 20H, 10 x CH_2 aliphatics), 0.96 (s), 0.92 (s), 0.91 (m, 9H, 3 x CH_3), 0.85 (t, J = 6.8 Hz, 3H, CH_3).

Benzyl (2R,4R)-2-(Tert-butyl)-3-formyl-4-(decanoyl)-oxazolidine-4-carboxylate (6Bb). Compound **6Bb** was prepared according to the above given procedure for compound **6Aa**. Compound **6Bb** was isolated in a yield of 63.8% as a viscous oil, having R_F 0.54 in petroleum ether/ethyl acetate (4:1); $[\alpha]_D^{20}$ = -2.85° (*c* = 1.47, $CHCl_3$); ^1H NMR ($CDCl_3$, 250 MHz): 8.31, 8.25 (2s, 1H, formyl proton), 7.36 (m, 5H, aromatic protons), 5.46, 5.05 (2s, 1H, H-C(2)), 5.29 (m, 2H, benzylic protons), 4.65 (dd, J = 10.2 , 9.1 Hz, 1H, H-C(5)), 4.11 (dd, J = 10.2, 9.1 Hz, 1H, H-C(5)), 2.59 (dt, J = 10.2, 7.6 Hz), 2.41 (m, 2H, 2H-C(2')), 1.55 (m, 2H, 2H-C(3')), 1.24 (m, 12 H, 6 x CH_2 aliphatics), 0.91, 0.88 (2s, 9H, 3 x CH_3), 0.85 (t, J = 6.6 Hz, 3H, CH_3).

Anal. Calcd for $C_{26}H_{39}NO_5$: C, 70.08; H, 8.82; N, 3.14. Found; C, 70.07; H, 8.69; N, 3.15.

Methyl (2R)-2-Amino-2-hydroxymethyl-3-ketooctadecanoate hydrochloride (*R*-7Aa). To a solution of compound **6Aa** 2.0 g (4.4 mmol) in anhydrous methanol (15 mL) is added under nitrogen atmosphere 5 mL of concd. HCl dropwise, with stirring at room temperature. The reaction mixture is stirred at room temperature for 48 h, the solvents removed under reduced pressure and the residue was dissolved in 1,4-di-

oxan (5 mL). The residue was cooled the residue in liquid nitrogen and lyophilised. This gave 1.73 g of the crude reaction product as a white powder. 0.717 g of this powder was dissolved in benzene (4 mL) and kept in a refrigerator. After 24 h, the product was centrifuged and the solvent removed. The procedure was repeated and gave 0.435 g (74.6%) of the title product, as a white powder, having mp: 108-9 ^{o}C; $[\alpha]_D^{20}$ = +35.6^{o} (*c* = 0.98, $CHCl_3/CH_3OH$ (1:1)); ^{1}H NMR (400 MHz, DMSO-d^6): 8.89 (br s, 3H, $^+NH_3$), 6.07 (br s, 1H, OH), 4.15, 4.10 (dd, J = 11.5 Hz, 2H, 2H-C(1)), 3.81 (s, 3H, OCH_3), 2.76 (m, 2H, 2H-C(4)), 1.48 (m, 2H, 2H-C(5)), 1.24 (m, 24H 12 x CH_2 aliphatics), 0.86 (t, J = 6.3 Hz, 3H, CH_3).

Anal. Calcd for $C_{20}H_{40}ClNO_4$: C, 60.97; H, 10.23; N, 3.55. Found: C, 60.98; H, 10.39; N, 3.40.

Methyl (2R)-2-Amino-2-hydroxymethyl-3-ketododecanoate hydrochloride (*R*-**7Ab**). Compound *R*-**7Ab** was prepared according to the above given procedure for compound *R*-**7Aa**. Compound *R*-**7Ab** was obtained in a yield of 89.5% as a semi-solid material, having $[\alpha]_D^{20}$ = +45.33^{o} (*c* = 3.34, $CHCl_3/CH_3OH$ (1:1); ^{1}H NMR ($CDCl_3$, 250 MHz): 8.80 (br s, 3H, $^+NH_3$), 4.52 (dd, J = 13.2 Hz, 2H, 2H-C(1)), 3.91 (s, 3H, OCH_3), 2.80 (m, 2H, 2H-C(4)), 1.59 (m, 2H, 2H-C(5)), 1.25 (m, 12 H, 6 x CH_2 aliphatics), 0.88 (t, J = 6.3 Hz, 3H, CH_3).

Anal. Calcd for $C_{14}H_{28}ClNO_4$: C, 54.27; H, 9.11; N, 4.52. Found: C, 54.83; H, 9.37; N, 4.60.

Methyl (2R,4E)-2-Amino-2-hydroxymethyl-3-keto-4-octadecenoate hydrochloride (*R*-**7Ac**). Compound *R*-**7Ac** was prepared, according to the above given procedure for compound *R*-**7Aa**. Compound *R*-**7Ac** was obtained in a yield of 43.2% as a powder, $[\alpha]_D^{20}$ = +55^{o} (*c* = 0.08, CH_3OH); ^{1}H NMR ($CDCl_3$, 250 MHz): 6.90 (dt, J = 15.2, 7.5 Hz, 1H, H-C(5)), 5.86 (d, J = 15.2 Hz, 1H, H-C(4)), 4.0 (m, 2H, 2H-C(1)), 3.81 (s, 3H, OCH_3), 2.4 (br s, 1H, OH), 2.18 (m, 2H, 2H-C(6)), 1.45 (m, 2H, 2H-C(7)), 1.26 (m, 20H, 10 x CH_2 aliphatics), 0.88 (t, J = 6.3 Hz, 3H, CH_3).

Anal. Calcd for $C_{20}H_{38}ClNO_4$: C, 61.28; H, 9.77; N, 3.57. Found: C, 61.34; H, 9.22; N, 4.35.

Benzyl (2R)-2-Amino-2-hydroxymethyl-3-ketododecanoate hydrochloride (*R*-**7Bb**). Compound *R*-**7Bb** was prepared, according to the above given procedure for compound *R*-**7Aa**. Compound *R*-**7Bb** was obtained in a yield of 70% as a semi solid material, having $[\alpha]_D^{20}$ = +33.94° (*c* = 2.85, $CHCl_3/CH_3OH$ (1:1)); ^1H NMR ($CDCl_3$, 250 MHz): 8.85 (br s, 3H, $^+NH_3$), 7.35 (m, 5H, aromatic protons), 5.30 (s, 2H, benzylic protons), 4.52 (dd, J = 12.9 Hz, 2H, 2H-C(1)), 2.62 (t, J = 7.2 Hz, 2H-C(4)), 2.17 (br s, 1H, OH), 1.44 (m, 2H, 2H-C(5)), 1.21 (m, 12H, 6 x CH_2 aliphatics), 0.88 (t, J = 6.6 Hz, 3H, CH_3).

Anal. Calcd for $C_{20}H_{32}ClNO_4$: C, 62.24; H, 8.36; N, 3.63. Found: C, 60.99; H, 8.13; N, 3.97.

Methyl (2S,4R)-2-Tert-butyl-3-formyl-oxazolidine-4-carboxylate (ent-**4A**). Compound ent-**4A** was prepared, according to the above given procedure for compound **4B**. Compound ent-**4A** was isolated in an overall yield of 70.6% from **D**-serine, as colorless hexagonal crystals from ether/pentane, having mp 57.5-59 °C; R_F 0.51 in petroleum ether/ethyl acetate (1:1); $[\alpha]_D^{20}$ = +43° (*c* = 1.38, $CHCl_3$); ^1H NMR ($CDCl_3$, 250 MHz): 8.47, 8.38 (2s, 1H, formyl proton), 5.17, 4.88 (2s, 1H, H-C(2)), 4.90 (dd, J = 7.3, 3.0 Hz, 1H, H-C(4)), 4.48 (dd, J = 8.8, 3.0 Hz, 1H, H-C(5)), 4.03 (dd, J = 8.8, 7.3 Hz, 1H, H-C(5)), 3.80, 3.76 (2s, 3H, OCH_3), 0.97, 0.90 (2s, 9H, 3 x CH_3).

Anal. Calcd for $C_{10}H_{17}NO_4$: C, 55.80; H, 7.96; N, 6.51. Found: C, 55.52; H, 7.96; N, 6.58.

Methyl (2S,4S)-2-Tert-butyl-3-formyl-4-(hexadecanoyl)-oxazolidine-4-carboxylate (ent-**6Aa**). Compound ent-**6Aa** was prepared, according to the above given procedure for compound **6Aa**. Compound ent-**6Aa** was obtained in a yield of 63% from compound ent-**4A** as a colourless viscous oil, having an R_F 0.56 in petroleum ether/ethyl acetate (4:1); $[\alpha]_D^{20}$ = +2.23° (*c* = 4.3, $CHCl_3$); ^1H NMR ($CDCl_3$, 250 MHz): 8.32, 8.25 (2s, 1H, formyl proton), 5.48, 5.08 (2s, 1H, H-C(2)),

4.67 (dd, J = 10.2, 9.2 Hz, 1H, H-C(5)), 4.12 (dd, J = 10.2, 9.2 Hz, 1H, H-C(5)), 3.88, 3.85 (2s, 3H, OCH_3), 2.68, 2.51 (2dt, J = 10.2, 7.6 Hz, 2H, 2H-C(2')), 1.60 (m, 2H, 2H-C(3')), 1.25 (m, 24H, 12 x CH_2 aliphatics), 0.98, 0.94 (2s, 9H, 3 x CH_3), 0.88 (t, J = 6.3 Hz, 3H, CH_3).

Anal. Calcd for $C_{26}H_{39}NO_5$: C, 68.83; H, 10.44; N, 3.08. Found: C, 69.08; H, 10.53; N, 3.0.

Methyl (2S,4S)-2-Tert butyl-3-formyl-4-(decanoyl)-oxazolidine-4-carboxylate (ent-**6Ab**). Compound ent-**6Ab** was prepared, according to the above given procedure for compound **6Aa**. Compound ent-**6Ab** was obtained in a yield of 69% from compound ent-**4A** as a colourless thick oil, having an R_F 0.52 in petroleum ether/ethyl acetate (4:1) and $[\alpha]_D^{20}$ = +3.73° (*c* = 3, $CHCl_3$); ^1H NMR ($CDCl_3$, 250 MHz): 8.32, 8.25 (2s, 1H, formyl proton), 5.48, 5.08 (2s, 1H, H-C(2)), 4.67 (dd, J = 10.2, 9.1 Hz, 1H, H-C(5)), 4.11 (dd, J = 10.2, 9.1 Hz, 1H, H-C(5)), 3.89, 3.85 (2s, 3H, OCH_3), 2.66, 2.51 (2 dt, J = 10.2, 7.4 Hz, 2H, 2H-C(2')), 1.60 (m, 2H, 2H-C(3')), 1.26 (m, 12 H, 6 x CH_2 aliphatics), 0.98, 0.94 (2s, 9H, 3 x CH_3), 0.88 (t, J = 6.3 Hz, 3H, CH_3).

Anal. Calcd for $C_{20}H_{35}NO_5$: C, 65.01; H, 9.54; N, 3.79. Found: C, 65.56; H, 9.71; N, 3.50.

Methyl (2S)-2-Amino-2-hydroxymethyl-3-ketooctadecanoate hydrochloride (*S*-**7Aa**). Compound *S*-**7Aa** was prepared, according to the above given procedure for compound *R*-**7Aa**. Compopund *R*-**7Aa** was obtained in a yield of 79.3% from compound ent-**6Aa** as white powder, mp 97-99 °C; $[\alpha]_D^{20}$ = -33.44° (*c* = 1.88, $CHCl_3/CH_3OH$ (1:1)); ^1H NMR ($CDCl_3$, 250 MHz) 8.6 (br s, 3H, $^+NH_3$), 4.52 (m, 2H, 2H-C(1)), 3.91 (s, 3H, OCH_3), 2.78 (m, 2H, 2H-C(4)), 1.59 (m, 2H, 2H-C(5)), 0.88 (t, J = 6.3 Hz, 3H, CH_3).

Anal. Calcd for $C_{20}H_{40}ClNO_4$: C, 60.97; H, 10.23; N, 3.55. Found: C, 60.40; H, 10.46; N, 3.52.

Methyl (2S)-2-Amino-2-hydroxymethyl-3-ketododecanoate-hydrochloride (*S*-**7Ab**). Compound *S*-**7Ab** was prepared, according to the above given procedure for compound *R*-**7Aa**. Compound *S*-**7Ab** was obtained in a yield of 85% as a semi so-

lid material, having $[\alpha]_D^{20}$ = -43.20° (*c* = 2.22, $CHCl_3/CH_3OH$ (1:1); ^1H NMR ($CDCl_3$, 250 MHz): 8.79 (br s, 3H, $^+NH_3$), 4.50 (m, 2H, 2H-C(1)), 3.92 (s, 3H, OCH_3), 2.79 (m, 2H, 2H-C(4)), 1.59 (m, 2H, 2H-C(5)), 1.25 (m, 12H, 6 x CH_2 aliphatics), 0.88 (t, J = 6.3 Hz, 3H, CH_3).

Anal. Calcd for $C_{14}H_{28}ClNO_4$: C, 54.27; H, 9.11; N, 4.52. Found: C, 53.79; H, 9.06; N, 4.50.

Methyl (2R)-2-Hexadecanoylamino-2-hydroxymethyl-3-keto-octadecanoate (*R*-**8**). To a solution of compound *R*-**7Aa** 220 mg (0.56 mmol) and palmitic acid 144 mg (0.56 mmol) in anhydrous THF/CH_3OH (5:1) 12 mL, was added EEDQ (*N*-ethoxy-carbonyl-2-ethoxy-1,2-dihydroquinoline) 140 mg (0.56 mmol). The mixture was stirred at room temperature for 48 h. After evaporation of solvents, the residue was subjected to chromatography on silica gel using petroleum ether/ethyl acetate (9:1) as eluants. This gave 160 mg (50%) of the title product as a solid, mp 72-73 °C, having an R_F 0.46 in petroleum ether/ethyl acetate (4:1), $[\alpha]_D^{20}$ = -1.65° (*c* = 0.55, $CHCl_3$); ^1H NMR ($CDCl_3$, 250 MHz): 6.62 (d, 1H, NH), 5.26 (d, J = 6.5 Hz, 1H, OH), 4.12 (dd, J = 7.2 Hz, 2H, 2H-C(1)), 3.80 (s, 3H, OCH_3), 2.69 (m, 2H, 2H-C(4)), 2.27 (m, 2H, 2H-C(2')), 1.60 (m, 4H, 2H-C(5), 2H-C(3')), 1.25 (m, 48H, 24 x CH_2 aliphatics), 0.88 (t, J = 6.3 Hz, 6H, 2 x CH_3).

Anal. Calcd for $C_{36}H_{69}NO_5$: C, 72.55; H, 11.67; N, 2.35. Found: C, 72.68; H, 11.68; N, 2.33.

Methyl 2-Hexadecanoylamino-3-ketooctadecanoate (**9**). To a solution of compound *R*-**7Aa** 100 mg (0.25 mmol) in 5 mL THF was added a 50% solution of sodium acetate in water (5 mL). The solution was stirred vigorously and added 68.7 mg (0.25 mmol) of palmitoyl chloride in 2 mL THF. The reaction mixture was stirred for 24 h. The product was extracted with THF (200 mL), the organic layer washed with satd. NaCl (25 mL), dried ($MgSO_4$) and concentrated. The residue thus obtained was subjected to column chromatography over silica gel using petroleum ether/ethyl acetate (9:1) as eluents, and gave 60 mg (42%) of the title product, as a solid, mp

65-66 °C, having an R_F 0.55 in petroleum ether/ethyl acetate (4:1); ^1H NMR ($CDCl_3$, 250 MHz): 6.58 (d, J = 6.3 Hz, 1H, NH), 5.23 (d, J = 6.3 Hz, 1H, H-C(2)), 3.78 (s, 3H, OCH_3), 2.68 (m, 2H, 2H-C(4)), 2.25 (t, J = 7.3 Hz, 2H, 2H-C(2'), 1.59 (m, 4H, 2H-C(4), 2H-C(4')), 1.23 (m, 48H, 24 x CH_2 aliphatics), 0.86 (t, J = 6.3 Hz, 6H, 2 x CH_3).

Anal. Calcd for $C_{35}H_{67}NO_4$: C, 74.28; H, 11.93; N, 2.47. Found: C. 74.0; H, 11.81; N, 2.65.

Methyl (2R,4S)-2-tert-butyl-3-formyl-4-(1-hydroxy-1-dodecyl)-oxazolidine-4-carboxylate (10). A solution of compound **4A** (1.00 g, 4.65 mmol) in THF (6 mL) was added dropwise to a freshly prepared solution of LDA (5.12 mmol) in 35 mL THF/hexane (10:1) at -78°C. After stirring for 15 min a solution of laurinaldehyde (11.62 mmol) in THF (6 mL) was added. Stirring was continued for 3 h at -78°C and the reaction quenched with a solution of glacial acetic acid (1 mL) in diethyl ether (3 mL). The reaction mixture was poured into a half saturated aqueous solution of $NaHCO_3$ (80 mL) and extracted with diethyl ether (350 mL). The organic layer was dried (Na_2SO_4), the solvent was removed under reduced pressure and the residue was chromatographed on a column of silica gel (hexane/ethyl acetate, 3:1) to yield 260 mg (14%) of a colourless oil with R_F 0.28. Compound **10** was obtained as a mixture of diastereomers in the ratio of 9:1 (evaluated from the integration ratio of the *N*-formyl-signals in the ^1H NMR spectrum). ^1H NMR ($CDCl_3$, 200 MHz): 8.44, 8.37, 8.11, 8.07 (4s, 1H, formyl proton, ratio of diastereomers 9:1, ratio of rotamers 12:1 and 2:1), 5.4-4.8 (m, 1H), 4.5-3.4 (m, 5H), 1.8-1.1 (m, 24H), 1.15-0.8 (m, 10H).

Anal. Calcd for $C_{22}H_{41}NO_5$: C, 66.13; H 10.34; N, 3.50; Found: C, 66.26; H 10.57; N, 3.71.

2-Amino-2-Hydroxymethyl-3-hydroxy-tetradecanoic acid (11). A solution of compound **10** (200 mg, 0.5 mmol) in 6 N methanolic HCl (20 mL) was refluxed for 1 h. The solution was passed through Dowex 50 W X-4 (H^+) and the product was eluted with methanolic ammonia. The solvent was removed un-

der reduced pressure to give compound **11** (50.6 mg, 35%) as an oil. R_F (nBuOH:HOAc:H_2O = 2:1:1) = 0.72; ^1H NMR ($CDCl_3$, 200 MHz): 0.81-0.91 (m, 3H, $-CH_3$), 1.15-1.65 (m, 2H, $-(CH_2)_{10}-$), 3.78-4.15 (m, 5H, $-CH_2OH$, $-CH-OH$), 4.70-5.30 (s, br, 2H, $-NH_2$). FAB-MS: 289, 288.

Anal. Calcd for $C_{15}H_{31}NO_4 \cdot 1/2\ H_2O$: C, 60.37; H, 10.81; N, 4.69. Found: C, 60.16; H, 10.51; N, 4.84.

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