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A General Approach to the Enantiomeric Synthesis of Lipidic α-Amino Acids, Peptides and Vicinal Amino Alcohols

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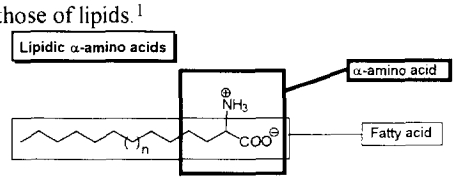
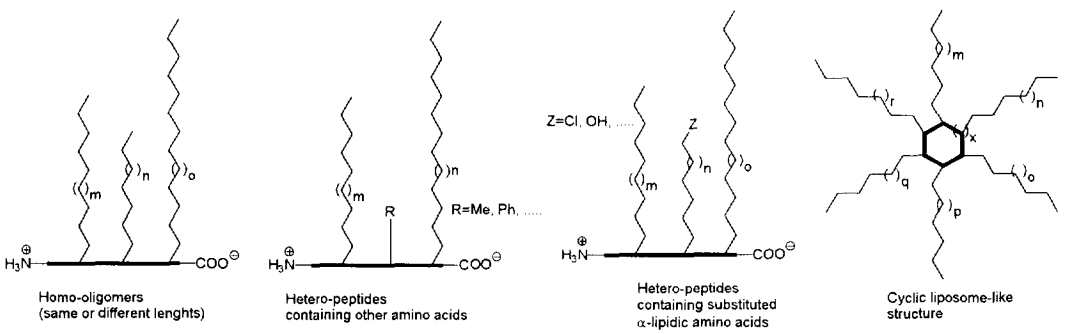
Abstract: A general methodology for the synthesis of saturated lipidic amino acids based on the oxidative cleavage of amino diols obtained by the regioselective opening of enantiomerically enriched 2,3-epoxy alcohols is described. The method opens the way to the synthesis of the enantiomers of lipidic 2-amino alcohols and homo- and hetero-peptides. Copyright © 1996 Elsevier Science Ltd

The α-lipidic amino acids (LAAs), non-natural α-amino acids with long alkyl side chains, and their homo-oligomers, the lipidic peptides, represent a class of compounds which combine structural features of amino acids and peptides with those of lipids.[1]

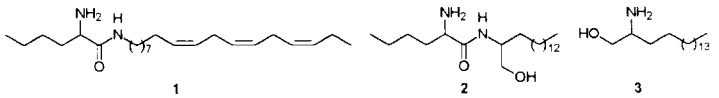


The amino acids can be linked to form peptides. The peptides can take up several forms. These include the linear homo-oligomers, hetero-peptides containing other amino acids or substituted lipidic amino acids or forming cyclic liposome-like structures. The length of the alkyl chains can be varied or substituted with other functional groups and the number of the α-amino acid residues in the peptide changed. This will affect the hydrophilic/lipophilic character of the lipidic peptides. The physical properties can then be expected to be highly lipophilic due to the long alkyl chains and yet exhibit polarity and conformations characteristic of amino acids and peptides.



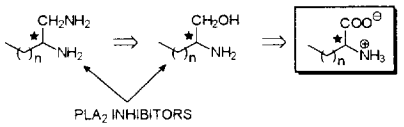
The potential use of lipidic amino acids is wide: lubricants,[2] polishes,[3] cosmetics,[4] surface improvers for ceramics (weatherproof coatings),[5] etc.. However, of particular interest for us is their use as a drug

delivery system,[6] as an adjuvant/carrier system[7] and as starting material for the synthesis of biologically interesting compounds such as sphingonine and ceramide analogs and lipidic 1,2-diamines.[8] Racemic lipidic α -amino acid amides and lipidic dipeptide derivatives have been found to inhibit both pancreatic and human platelet phospholipase (PLA_2) (compound **1** 11 μM, compound **2** IC_{50} 12 μM for pancreatic PLA_2).[9] In addition, lipidic 1,2-amino alcohols and 1,2-diamines in the racemic form exhibit potent inhibitory activity against PLA_2 (compound **3** IC_{50} 4 μM).[10]



1 **2** **3**

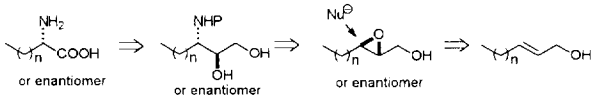
As all the above mentioned compounds can be obtained from lipidic amino acids, we planned the enantioselective synthesis of such compounds in a general way in order to be able to control size and stereochemistry in the final compounds.



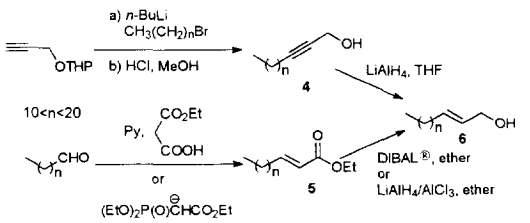
Racemic LAAs have been prepared by reacting α-bromoalkanoic acids with ammonium hydroxide[11] or 1-bromoalkanes with dialkyl acetamidomalonate, followed by hydrolysis, partial decarboxylation of the intermediate.[1,12] The enzymatic or chemical resolution of the obtained racemic product has been used to obtain the enantiomers.[1]

We present in this paper a general approach to the enantiomeric synthesis of lipidic 3-amino-1,2-diols,[13] 2-amino alcohols, α-amino acids and homo- and hetero-peptides based on the regioselective opening of chiral 2,3-epoxy alcohols.[14] For this approach we must focus our attention on two major points:

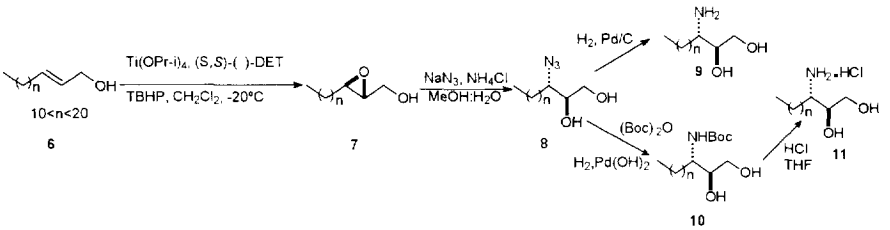
1. The synthesis of the suitable precursor. In our case, the allylic alcohol necessary to perform the asymmetric epoxidation using the proper chiral auxiliary. In our case, the choice of (*R*,*R*) or (*S*,*S*)-dialkyl tartrates, depending on which final enantiomer of the α-amino acid we are going to prepare.
2. The choice of the appropriate nucleophile containing nitrogen. It should be selected taking into consideration regioselectivity and yield of the opening reaction as well as facilities to transform such a group in the final amino group. Also the choice of the nucleophile should be made considering the necessary cleavage of the C-C bond in order to obtain the acid group.



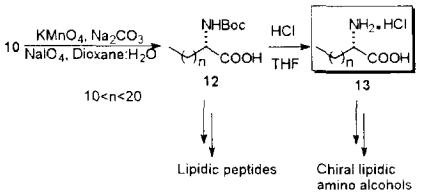
With this idea in mind we synthesized the necessary allylic alcohol **6**. Two main methodologies have been used. The first one is based on the alkylation of protected propargyl alcohol and stereoselective reduction of the free propargylic alcohol using $LiAlH_4$. The second one use either a Wittig-Horner reaction[15c] or a Knoevenagel condensation over a suitable linear aldehyde to obtain the *E*-unsaturated ester which is reduced with DIBAL® or AlH_3 to the desired allylic alcohol. Considering that the propargylic approach produces one homologation of three carbon atoms and the Wittig-Horner or Knoevenagel approach extends the chain by two carbons, both methods are based on economic reasons considering the relative price of the precursor alkyl bromide or aldehyde.



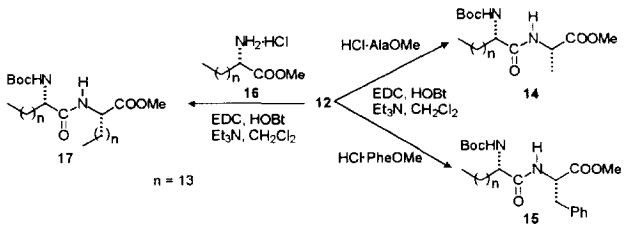
The allylic alcohols **6** were submitted to Sharpless asymmetric epoxidation with the expected yields and ee's (>80% yield and >95% ee).[15] The only consideration that should be made in this reaction is that because of the low solubility in CH_2Cl_2 of large allylic alcohols **6,** the addition of such a precursor should be slow enough to avoid precipitation that can dramatically decrease the enantiomeric purity and yield of the obtained epoxides **7**. Epoxides opening using sodium azide and ammonium chloride yielded the azido diol **8** with good regioselectivity (>10:1) and yield.[14a,16] The reduction of the azide under standard conditions yielded the corresponding amino diols **9** which interestingly have shown very good activity against pancreatic PLA_2(IC_{50} 3-4 μM).[13] This direct reduction led, however, to a poor yield of **9**. As an alternative approach we found that the concomitant reduction of the azido group and N-Boc protection[17] was more convenient either to obtain directly the N-Boc protected amino diols **10** or the amino alcohol hydrochlorides **11** since both steps are practically quantitative.



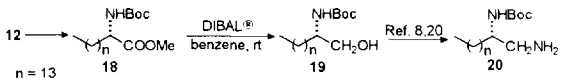
Finally, when **10** were submitted to oxidative cleavage using potassium permanganate[18] the N-Boc protected amino acids **12** were obtained with excellent yields (>85%). Final deprotection of the Boc-group under acidic conditions yielded the lipidic α-amino acids **13**.



In order to explore the scope and limitations of the use of chiral lipidic aminoacids in peptide chemistry, we attempted the synthesis of both homo- and hetero-peptides. Thus, the N-Boc-protected amino acid **12** (n = 13) was successfully coupled with the methyl ester hydrochlorides of both L-alanine and L-phenylalanine, using N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) as water soluble peptide coupling reagent,[19] yielding the protected hetero-peptides **14** and **15** in high yield. In the same manner, **12** was coupled with the methyl ester **16** yielding the protected lipidic homo-peptide **17**.



In the series of peptides shown above only one diastereoisomer was detected in each case showing that the optical purity of the obtained lipidic amino acids should be at least similar to the starting epoxy alcohol. In order to confirm such purity we transformed **12** (n = 13) into the corresponding methyl ester **18** which was reduced with DIBAL® in benzene at room temperature, yielding the N-Boc-1,2-amino-alcohol **19** in high yield. The preparation of the corresponding (+) and (−)-Mosher esters and 400 MHz ^{1}H NMR analysis showed an optical purity of more than 95% ee. This method can be then considered to be a general methodology to obtain lipidic 2-amino-1-alcohols **19** and 1,2-diamines **20**,[8,20] both of which interestingly also showed strong activity as PLA_2 inhibitors.[10] Although the presented methodology have been described only for one enantiomer series the choice of the proper stereoisomer of the epoxy alcohol of **7** permits the control of the absolute configuration in the final products.



Experimental Section

Materials and Methods. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX 400 and/or Bruker AC 200 spectrometer in $CDCl_3$ or CD_3OD as solvent, and chemical shifts are reported relative to Me_4Si. Low-resolution mass spectra was taken using a Hewlett-Packard Model 257. Elemental analyses were performed on a Carlo-Erba Model 1106. Optical rotations were determined for solutions in chloroform, methanol or benzene with a Perkin-Elmer Model 241 polarimeter. Infrared spectra were recorded on a Bruker Model IFS55. GC analyses were performed on a Hewlett-Packard HP-5890 instrument with a capillary column, OV-1, 25 m. Melting points were determined on a Büchi model 535 melting point apparatus and are uncorrected. HPLC chromatography was performed using a LKB PUMP Model 2248 with a LKD 2MD RAPID SPECTRAL detector using a μ-Porasil Silica 10 μm WATERS column. Column chromatography was performed on silica gel, 0.015-0.04 and 0.04-0.063 mm, and TLC and PLC were performed on silica gel, all Merck products. Visualization of spots was effected with UV light and/or phosphomolybdic acid and/or ninhydrin both in ethanol stain. All solvents were purified by standard techniques. Reactions requiring anhydrous conditions were performed under argon. Anhydrous magnesium sulfate was used for drying solutions.

General Method for the Alkylation of Protected Propargyl Alcohols with Long Chain Halides. Preparation of Heptadec-2-yn-1-ol (4). To a stirred solution of propargyl-OTHP ether (10 g, 71.4 mmol) in dry THF (350 mL) under argon was added dropwise *n*-BuLi (59.5 mL, 1.2 M solution in *n*-hexane, 75.4 mmol) at -78 °C. The reaction was allowed to warm to rt. After cooling again at -78 °C, HMPA (12.5 mL, 71.4 mmol) and 1-bromotetradecane (myristyl bromide) (23.4 mL, 78.5 mmol) were added dropwise. The reaction mixture was allowed to warm until rt and stirred overnight. After this period TLC showed complete

conversion. Then the reaction mixture was quenched with a saturated aqueous solution of NH_4Cl (200 mL). The organic phase was washed with brine (200 mL), dried over $MgSO_4$, filtered and the solvent was evaporated. The crude mixture was dissolved in methanol (350 mL) and concentrated HCl was catalytically added until pH 1. The reaction mixture was monitored by TLC and after 5 min there was complete conversion. Et_3N was added until pH 9 and the reaction mixture was stirred for 5 min. After evaporation of the solvent, the crude was purified by silica gel column chromatography to afford **4** (15.3 g, 85% yield) as a white solid: mp = 51 °C; ^1H NMR ($CDCl_3$) δ : 0.88 (t, J = 6.6 Hz, 3H), 1.26 (br s, 20H), 1.36 (m, 2H), 1.51 (ddd, J = 14, 14, 6 Hz, 2H), 1.61 (s, 1H, OH), 2.21 (dddd, J = 9.2, 9.2, 4.2, 2.1 Hz, 2H), 4.25 (t, J = 2.5 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ : 14.1 (q), 18.7 (t), 22.7 (t), 28.6 (t), 28.9 (t), 29.1 (t), 29.1 (t), 29.3 (t), 29.5 (t), 29.5 (t), 29.6 (t), 29.6 (t), 29.6 (t), 31.9 (t), 51.43 (t), 78.24 (s), 86.7 (s); IR ($CHCl_3$) (cm^{-1}) 3610, 2928, 2855, 2219, 1466, 1381, 1136, 1004, 961; MS *m/z* (relative intensity) 221 $(M - CH_2OH)^+$ (2), 135 (14), 121 (24), 111 (37), 107 (22), 97 (30), 93 (63), 83 (79), 79 (78), 70 (93), 67 (97), 55 (100). Anal. Calcd. for $C_{17}H_{32}O$: C, 80.89; H, 12.78. Found: C, 80.84; H, 12.89.

General Method for the Preparation of Long Chain *E*-α-β-unsaturated Esters by the Knoevenagel Approach. Preparation of Ethyl Hexadec-2*E*-enoate (5). To a solution of commercially available 1-tetradecanol (1 g, 4.7 mmol) in dry CH_2Cl_2 (25 mL) under argon were sequentially added DMSO (3 mL, 0.66 mL x mmol), Et_3N (3.3 mL, 23.5 mmol) and SO_3·py (2.2 g, 14.1 mmol) at rt. The mixture was stirred and after 30 min TLC showed complete conversion. Then to the reaction mixture was added 5% HCl aqueous solution (20 mL) and it was extracted with CH_2Cl_2 (2 x 10 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated. To the crude were sequentially added pyridine (0.5 mL, 5.6 mmol), hemimalonate ethyl ester (0.7 mL, 5.2 mmol) and a catalytic amount of piperidine (2 drops). This mixture was heated for 2 hours in a water bath at 60 °C until TLC showed complete conversion. The mixture was diluted with Et_2O (30 mL) and washed with 5% HCl aqueous solution. The organic layer was dried over $MgSO_4$, filtered, concentrated and purified by silica gel column chromatography to afford **5** (1 g, 80% yield) as an oil: ^1H NMR ($CDCl_3$) δ : 0.88 (t, J = 6.5 Hz, 3H), 1.26 (br s, 20H), 1.28 (t, J = 7.1 Hz, 3H), 1.45 (dd, J = 7, 7 Hz, 2H), 2.18 (ddd, J = 7.7, 7.7, 7.7 Hz, 2H), 4.18 (q, J = 7.1 Hz, 2H), 5.80 (d, J = 15.6 Hz, 1H), 6.96 (ddd, J = 15.6, 7, 7 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ : 14.0 (q), 14.2 (q), 22.6 (t), 28.0 (t), 29.1 (t), 29.2 (t), 29.3 (t), 29.4 (t), 29.5 (t), 29.6 (t), 29.7 (t), 29.8 (t), 31.9 (t), 32.1 (t), 60.0 (t), 121.2 (d), 149.4 (d), 166.7 (s); IR ($CHCl_3$) (cm^{-1}) 3020, 2929, 2855, 1708, 1652, 1466, 1370, 1311, 1271, 1187, 1042, 981; MS *m/z* (relative intensity) 283 $(M + 1)^+$ (78), 236 (18), 194 (12), 155 (13), 141 (18), 127 (16), 110 (18), 101 (53), 96 (50), 88 (38), 69 (44), 55 (100). Anal. Calcd. for $C_{18}H_{34}O_2$: C, 76.54; H, 12.13. Found: C, 76.25; H, 12.39.

General Method for the Reduction of Long Chain Propargylic Alcohols to *E*-allylic alcohols. Preparation of Heptadec-2*E*-en-1-ol (6). Method i. To a stirred solution of ethyl heptadec-2*E*-enoate (1 g, 3.4 mmol) in Et_2O (320 mL) in an ice-cold bath was added slowly DIBAL® (37 mL, 1.0 M in hexane, 37 mmol). After 5 min to the reaction mixture H_2O (0.5 mL), 15% aqueous NaOH solution (0.5 mL) and H_2O (1.5 mL) were sequentially added with stirring. The mixture was allowed to reach rt, dried over $MgSO_4$, filtered through a pad of celite, concentrated, and purified by silica gel column chromatography, to yield **6** (756 mg, 88% yield) as a solid.

Method ii. To a stirred solution of propargyl alcohol **4** (10 g, 39.6 mmol) in ether (350 mL) in an ice-cold bath was added slowly $LiAlH_4$ (43.2 mL, 0.55 M in Et_2O, 23.7 mmol). After 1 h to the reaction mixture H_2O (0.9 mL), 15% NaOH aqueous solution (0.9 mL) and H_2O (2.7 mL) were sequentially added with stirring. The mixture was allowed to reach rt, dried over $MgSO_4$, filtered through a pad of celite,

concentrated, and purified by silica gel column chromatography, to yield **6** (8 g, 80% yield) as a solid: mp = 40 °C; ^{1}H NMR ($CDCl_3$) δ: 0.88 (t, J = 7 Hz, 3H), 1.26 (br s, 22H), 1.36 (m, 2H), 1.59 (s, 1H, OH), 2.05 (m, 2H), 4.09 (d, J = 5.3 Hz, 2H), 5.67 (m, 2H); ^{13}C NMR ($CDCl_3$) δ: 14.1 (q), 22.7 (t), 29.1 (t), 29.2 (t), 29.3 (t), 29.5 (t), 29.6 (t), 29.7 (t), 29.7 (t), 29.7 (t), 29.7 (t), 29.7 (t), 31.9 (t), 32.2 (t), 63.9 (t), 128.8 (d), 133.6 (d); IR ($CHCl_3$) (cm^{-1}) 3612, 3019, 2927, 2855, 1459, 1084, 972; MS *m*/z (relative intensity) 236 (M $-H_2O)^+$ (1), 125 (3), 111 (10), 97 (24), 83 (46), 81 (48), 71 (34), 69 (52), 57 (100), 55 (96). Anal. Calcd. for $C_{17}H_{34}O$: C, 80.24; H, 13.47. Found: C, 80.10; H, 13.68.

General Method for the Asymmetric Epoxidation of Long Chain Allylic Alcohols. Preparation of (2*S*,3*R*)-(3-Tetradecyl-oxiranyl)-methanol (7). Crushed, activated 3Å molecular sieves (20% w) were added to stirred CH_2Cl_2 (100 mL) under argon. The flask was cooled to -20 °C and Ti(OPr-*i*)$_4$ (4.3 mL, 14.4 mmol), (S,S)-(−)-diethyl tartrate (2.9 mL, 16.8 mmol), and *tert*-butyl hydroperoxide (4.4 mL, 5.5 M solution in *iso*-octane, 24 mmol) were added sequentially with stirring. The mixture was stirred for 15 min, and was added *slowly dropwise* heptadec-2*E*-en-1-ol **6** (3 g, 12 mmol) dissolved in CH_2Cl_2 (20 mL). After the addition, the reaction was maintained with stirring for 4 h. Tartaric acid aqueous solution (15% w/v, 100 mL) was added, and the stirring was continued until clear phases were reached (30 min). The phases were separated, and the aqueous phase was extracted with CH_2Cl_2 (2 x 30 mL). The combined organic phases were concentrated, diluted with ether (50 mL), and treated with an ice-cold 15% aqueous solution of NaOH (w/v) (50 mL). The two-phase mixture was stirred vigorously for 15 min at 0 °C. The organic phase was separated, and the aqueous phase extracted with ether (2 x 20 mL). The combined organic phases were washed with brine (50 mL), dried over $MgSO_4$, filtered, evaporated and purified by silica gel column chromatography, to yield **7** (2.9 g, 89% yield, > 95% ee by ^{1}H-NMR analysis of the Mosher's ester[21]): mp = 77 °C; $[\alpha]_D^{25}$ +18.2 (*c* 2.0, $CHCl_3$) [lit.[22] $[\alpha]_D^{25}$ −27.0 (c 0.87, benzene) of the enantiomer]; ^{1}H NMR ($CDCl_3$) δ: 0.88 (t, J = 6.5 Hz, 3H), 1.26 (br s, 20H), 1.44 (ddd, J = 7.1, 7.1 Hz, 2H), 1.60 (m, 4H), 2.93 (dd, J = 8, 2 Hz, 1H), 2.96 (dd, J = 5.5, 2 Hz, 1H), 3.63 (ddd, J = 11.4, 11.4, 4.5 Hz), 3.91 (ddd, J = 12.4, 2.6, 2.6 Hz) ; ^{13}C NMR ($CDCl_3$) δ: 14.1 (q), 22.6 (t), 25.8 (t), 25.9 (t), 29.2 (t), 29.3 (t), 29.4 (t), 29.5 (t), 29.6 (t), 31.4 (t), 31.5 (t), 31.7 (t), 31.8 (t), 31.9 (t), 56.0 (d), 58.5 (d), 61.7 (t); IR ($CHCl_3$) (cm^{-1}) 3736, 2928, 2855, 1542, 1100, 1062; MS *m*/z (relative intensity) 239 (M $-CH_2OH)^+$ (3), 182 (1), 181 (1), 167 (1), 153 (2), 139 (4), 125 (12), 111 (38), 97 (91), 83 (97), 69 (99), 57 (100), 55 (100). Anal. Calcd. for $C_{17}H_{34}O_2$: C, 75.50; H, 12.67. Found: C, 75.61; H, 12.78.

General Method for the Epoxide Opening with NaN_3. Preparation of (2*S*,3*S*)-3-Azido-heptadecane-1,2-diol (8). To a solution of the epoxy alcohol **7** (2.8 g, 10 mmol) in an 8:1 MeOH:H_2O mixture (90 mL) were added NaN_3 (3.25g, 50 mmol) and NH_4Cl (1.2 g, 22 mmol) with stirring at rt. The reaction was then refluxed for 8 h until TLC showed complete conversion. The solvent was evaporated under vacuum, and the crude dissolved in AcOEt (50 mL) and washed with brine (50 mL). The aqueous phase was extracted with AcOEt (2 x 20 mL), the combined organic layers were dried over $MgSO_4$, filtered, the solvent was evaporated and the residue was purified by silica gel column chromatography, yielding **8** (2.7 g, 86% yield) as a white solid: mp = 47 °C; $[\alpha]_D^{25}$ +8.0 (*c* 2.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ: 0.88 (t, J = 6.4 Hz, 3H), 1.26 (br s, 22H), 1.56 (m, 4H), 1.86 (s, 1H, OH), 2.45 (s, 1H, OH), 3.45 (ddd, J = 8.8, 8.8, 4.4 Hz, 1H), 3.72 (dd, J = 16, 7 Hz, 2H), 3.83 (dd, J = 52, 4.4 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ: 14.1 (q), 22.7 (t), 26.3 (t), 29.4 (t), 29.5 (t), 29.5 (t), 29.6 (t), 29.6 (t), 29.6 (t), 29.6 (t), 29.6 (t), 29.7 (t), 30.6 (t), 31.9 (t), 63.1 (t), 64.7 (d), 73.5 (d); IR ($CHCl_3$) (cm^{-1}) 3567, 3406, 2928, 2855, 2106, 1466, 1267, 1067, 1032, 865; MS *m*/z (relative

intensity) 254 (M –OH, N_3)$^+$ (1), 224 (7), 125 (2), 111 (5), 97 (13), 83 (22), 69 (53), 61 (100), 57 (100), 55 (85). Anal. Calcd. for $C_{17}H_{35}N_3O_2$: C, 65.13; H, 11.25; N, 13.40. Found: C, 65.21; H, 11.28; N, 13.22.

General Method for the One-Pot Transformation of Azido Diols to N-Boc-amino Diols. Preparation of (2*S*,3*S*)-3-*t*-Butoxycarbonylamino-heptadecane-1,2-diol (10). To a solution of the azido diol **8** (1.78 g, 5.7 mmol) and Boc_2O (2.5 g, 11.4 mmol) in dry AcOEt (60 mL) was added $Pd(OH)_2$ (180 mg, 10% w) at rt. The resulting reaction mixture was stirred under hydrogen atmosphere at rt until disappearance of the azido diol as monitored by TLC diagnosis. The mixture was filtered through a Celite pad to eliminate the catalyst and concentrated. In order to separate the pure product from the unchanged Boc_2O the crude was purified by silica gel column chromatography, to yield **10** (2.2 g, 98% yield) as a white solid: mp = 83 °C; $[\alpha]_D^{25}$ –8.6 (*c* 2.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ : 0.88 (t, J = 6.6 Hz, 3H), 1.26 (br s, 24H), 1.45 (s, 9H), 1.75 (m, 1H), 1.85 (m, 1H), 2.83 (d, J = 8 Hz, 1H), 3.30 (br s, 2H, OH), 3.50 (d, J = 7.6 Hz, 1H), 3.61 (d, J = 7.1 Hz, 1H), 3.68 (m, 1H), 4.53 (d, J = 7.5 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ : 14.0 (q), 14.1 (q), 22.6 (t), 25.9 (t), 28.1 (t), 28.3 (t), 28.4 (t), 28.5 (t), 29.1 (t), 29.3 (t), 29.4 (t), 29.5 (t), 29.6 (t), 31.2 (t), 32.0 (t), 52.5 (d), 63.0 (t), 74.5 (d), 80.2 (s), 157.3 (s); IR ($CHCl_3$) (cm^{-1}) 3649, 3567, 3442, 2928, 2855, 1686, 1506, 1458, 1369, 1242, 1166, 1064, 1013, 862; MS *m*/z (relative intensity) 388 (M + 1)$^+$ (5), 332 (22), 288 (9), 270 (36), 226 (65), 87 (12), 69 (12), 60 (26), 57 (100). Anal. Calcd. for $C_{22}H_{45}NO_4$: C, 68.17; H, 11.70; N, 3.61. Found: C, 68.11; H, 11.77; N, 3.82.

General Method for N-Boc Cleavage. Preparation of (2*S*,3*S*)-3-Amino-heptadecane-1,2-diol Hydrochloride (11). The N-Boc-amino diol **10** (195 mg, 0.5 mmol) was treated with 4N HCl in THF (6.2 mL, 25 mmol) at rt until TLC showed complete deprotection. The excess acid and solvent were removed under reduced pressure and the residue was reevaporated twice from anhydrous THF (2 x 5 mL). The residue was triturated with dry Et_2O to afford **11** (160 mg, 98% yield) as a white solid: mp = 120 °C (dec.); $[\alpha]_D^{25}$ – 5.8 (*c* 1.8, MeOH); ^{1}H NMR (CD_3OD) δ : 0.85 (t, J = 6.4 Hz, 3H), 1.24 (br s, 24H), 1.44 (m, 1H), 1.64 (m, 1H), 3.26 (m, 1H), 3.59 (dd, J = 11.3, 5.3 Hz, 1H), 3.67 (dd, J = 11.3, 4.8 Hz, 1H), 3.75 (dd, J = 4.5, 4.5 Hz, 1H); ^{13}C NMR (CD_3OD) δ : 12.9 (q), 22.2 (t), 25.3 (t), 27.6 (t), 29.0 (t), 29.1 (t), 29.2 (t), 29.3 (t), 29.4 (t), 29.5 (t), 29.6 (t), 29.7 (t), 29.8 (t), 31.6 (t), 54.6 (d), 62.2 (t), 69.6 (d); IR (Nujol) (cm^{-1}) 3587, 3386, 3172, 2953, 2923, 2854, 2726, 2671, 1461, 1377, 1306, 1154, 1062, 972; MS *m*/z (relative intensity) 288 (M – HCl)$^+$ (9), 256 (13), 226 (100), 90 (11), 83 (10), 69 (17), 60 (82), 56 (69). Anal. Calcd. for $C_{17}H_{38}NO_2Cl$: C, 63.03; H, 11.82; N, 4.32. Found: C, 62.99; H, 12.01; N, 4.48.

General Method for the Oxidative Cleavage of Amino Diols. Preparation of (2*S*)-2-*t*-Butoxycarbonylamino-hexadecanoic Acid (12). The N-Boc amino diol **10** (780 mg, 2 mmol) was dissolved in a 2.3:1 dioxane:H_2O mixture (20 mL). Then $NaIO_4$ (1.7 g, 8 mmol), Na_2CO_3 (106 mg, 1 mmol) and $KMnO_4$ (64 mg, 0.4 mmol) were sequentially added. The reaction mixture was stirred until the pink color disappeared and a brown precipitate was formed. The reaction was completed at that time as shown on TLC. The reaction mixture was filtered through a pad of celite. Then the filtrate was acidified with 5% HCl solution and extracted with Et_2O (3 x 20 mL). The combined organic layers were dried over $MgSO_4$, filtered, evaporated and purified by silica gel column chromatography to afford **12** (700 mg, 95% yield) as a white solid: mp = 41 °C; $[\alpha]_D^{25}$ +7.9 (*c* 2.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ : 0.88 (t, J = 6.8 Hz, 3H), 1.26 (br s, 24H), 1.45 (s, 9H), 1.65 (m, 1H), 1.84 (m, 1H), 4.30 (br s, 1H), 5.02 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ : 14.0 (q), 14.1 (q), 22.6 (t), 25.5 (t), 28.3 (t), 29.0 (t), 29.2 (t), 29.3 (t), 29.4 (t), 29.5 (t), 29.6 (t), 29.7 (t), 29.8 (t), 31.8 (t), 32.4 (t), 53.4 (q), 54.6 (d), 80.0 (s), 155.6 (s), 177.6 (s); IR ($CHCl_3$) (cm^{-1}) 3741, 3443, 2928, 2855, 1710, 1506, 1457, 1394, 1369, 1165, 1051, 859; MS *m*/z (relative intensity) 372 (M + 1)$^+$ (1), 316 (16), 270

(36), 226 (88), 143 (13), 118 (11), 74 (17), 57 (100). Anal. Calcd. for $C_{21}H_{41}NO_4$: C, 67.88; H, 11.12; N, 3.77. Found: C, 67.59; H, 11.32; N, 3.52.

General Method for Amino Acids Coupling. Preparation of Methyl (2*S*)-2-[(2*S*)-2-*t*-Butoxycarbonylamino-hexadecanoylamino]-propionate (14). The N-Boc amino acid **12** (50 mg, 0.13 mmol) and L-alanine methyl ester hydrochloride (20 mg, 0.13 mmol) were dissolved in dry CH_2Cl_2 (1.3 mL) at rt. Then 1-hydroxybenzotriazole (23 mg, 0.13 mmol) and N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide hydrochloride (30 mg, 0.14 mmol) were added. To the reaction mixture was added Et_3N (drops) until pH 10. The mixture was stirred until TLC showed complete conversion. The solvent was evaporated and the residue was purified by silica gel column chromatography to afford **14** (50.1 mg, 84% yield) as a white solid: mp = 43 °C; $[\alpha]_D^{25}$ −4.1 (*c* 2.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ: 0.87 (t, J = 6.5 Hz, 3H), 1.24 (br s, 24H), 1.39 (d, J = 7.2 Hz, 3H), 1.43 (s, 9H), 1.58 (m, 1H), 1.79 (m, 1H), 3.74 (s, 3H), 4.06 (br s, 1H), 4.57 (dddd, J = 7.3, 7.3, 7.3, 7.3 Hz, 1H), 5.02 (br s, 1H), 6.62 (d, J = 5.6 Hz); ^{13}C NMR ($CDCl_3$) δ: 14.0 (q), 14.1 (q), 18.3 (q), 22.6 (t), 25.5 (t), 28.3 (t), 29.0 (t), 29.2 (t), 29.3 (t), 29.4 (t), 29.5 (t), 29.6 (t), 29.7 (t), 29.8 (t), 31.8 (t), 32.5 (t), 47.9 (d), 52.4 (q), 54.5 (d), 80.0 (s), 155.6 (s), 171.8 (s), 173.1 (s); IR ($CHCl_3$) (cm^{-1}) 3735, 3435, 2928, 2855, 1745, 1699, 1683, 1508, 1456, 1361, 1164, 1057, 974, 852; MS *m*/z (relative intensity) 457 $(M + 1)^+$ (2), 401 (6), 270 (51), 226 (72), 104 (12), 102 (17), 70 (14), 57 (100). Anal. Calcd. for $C_{25}H_{48}N_2O_5$: C, 65.75; H, 10.59; N, 6.13. Found: C, 67.65; H, 10.68; N, 6.20.

Preparation of Methyl (2*S*)-2-[(2*S*)-2-*t*-Butoxycarbonylamino-hexadecanoylamino]-3-phenyl-propionate (15). The general amino acids coupling was applied to **12** on a 50 mg scale (0.13 mmol) using L-phenylalanine methyl ester hydrochloride (30 mg, 0.13 mmol), 1-hydroxybenzotriazole (23 mg, 0.13 mmol) and N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide hydrochloride (30 mg, 0.14 mmol) to afford **15** (49.3 mg, 71% yield) as a white solid: mp = 68 °C; $[\alpha]_D^{25}$ +20.2 (*c* 2.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ: 0.87 (t, J = 6.5 Hz, 3H), 1.24 (br s, 24H), 1.43 (s, 9H), 1.52 (m, 1H), 1.76 (m, 1H), 3.07 (dd, J = 13.7, 6 Hz, 1H), 3.14 (dd, J = 13.7, 6 Hz, 1H), 3.69 (s, 3H), 4.02 (br s, 1H), 4.84 (dd, J = 13.7, 6 Hz, 1H), 4.93 (br s, 1H), 6.49 (d, J = 7.7 Hz, 1H), 7.10 (d, J = 6.7 Hz, 2H), 7.25 (m, 3H); ^{13}C NMR ($CDCl_3$) δ: 14.0 (q), 14.1 (q), 22.6 (t), 25.5 (t), 28.2 (t), 29.2 (t), 29.3 (t), 29.4 (t), 29.5 (t), 29.6 (t), 29.7 (t), 29.8 (t), 29.9 (t), 31.8 (t), 32.5 (t), 37.9 (t), 52.2 (q), 53.1 (d), 54.6 (d), 79.9 (s), 127.1 (d), 128.5 (d), 129.2 (d), 135.7 (s), 155.5 (s), 171.6 (s), 171.8 (s); IR ($CHCl_3$) (cm^{-1}) 3735, 3422, 2928, 2855, 1746, 1699, 1683, 1508, 1457, 1369, 1166, 1013, 852; MS *m*/z (relative intensity) 532 $(M)^+$ (1), 476 (6), 270 (31), 226 (52), 162 (20), 120 (13), 91 (15), 57 (100). Anal. Calcd. for $C_{30}H_{50}N_2O_5$: C, 69.46; H, 9.72; N, 5.40. Found: C, 69.56; H, 9.98; N, 5.58.

Preparation of Methyl (2*S*)-2-Aminohexadecanoate Hydrochloride (16). The general N-Boc cleavage was applied to **15** on a 195 mg scale (0.5 mmol) using a THF solution of HCl (4.8 mL, 5.2 N solution, 25 mmol) to afford **16** (160 mg, 98% yield) as a white solid: mp = 122 °C (dec.); $[\alpha]_D^{25}$ +14.2 (*c* 2.0, MeOH); ^1H NMR (CD_3OD) δ: 0.85 (t, J = 5.4 Hz, 3H), 1.24 (br s, 24H), 1.86 (m, 2H), 3.79 (s, 3H), 3.99 (t, J = 6.3 Hz, 1H); ^{13}C NMR (CD_3OD) δ: 15.4 (q), 24.7 (t), 26.9 (t), 31.2 (t), 31.3 (t), 31.4 (t), 31.5 (t), 31.6 (t), 31.7 (t), 31.8 (t), 31.9 (t), 32.5 (t), 34.1 (t), 54.6 (d), 55.0 (q), 172.1 (s); IR (Nujol) (cm^{-1}) 3417, 2952, 2921, 2853, 2726, 1745, 1594, 1461, 1377, 1305, 1234, 1167, 1062, 961; MS *m*/z (relative intensity) 286 $(M - HCl)^+$ (22), 226 (100), 97 (7), 88 (26), 83 (14), 69 (22), 56 (90). Anal. Calcd. for $C_{17}H_{36}NO_2Cl$: C, 63.43; H, 11.27; N, 4.35. Found: C, 62.13; H, 11.30; N, 4.12.

Preparation of Methyl (2*S*)-2-[(2*S*)-2-*t*-Butoxycarbonylamino-hexadecanoylamino]-hexadecanoate (17). The general amino acids coupling was applied to **12** on a 50 mg scale (0.13 mmol) using methyl (2S)-2-t-butoxycarbonylamino-hexadecanoate hydrochloride **16** (42 mg, 0.13 mmol), 1-hydroxybenzotriazole

(23 mg, 0.13 mmol) and N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide hydrochloride (30 mg, 0.14 mmol) to afford **17** (66 mg, 80% yield) as a white solid: mp = 66 °C; $[\alpha]_D^{25}$ -1.8 (*c* 2.2, $CHCl_3$); ^1H NMR ($CDCl_3$) δ: 0.87 (t, J = 6.6 Hz, 3H), 1.26 (br s, 24H), 1.43 (s, 9H), 1.61 (m, 1H), 1.75 (m, 1H), 3.72 (s, 3H), 4.27 (d, J = 5.8 Hz, 1H), 4.99 (m, 1H); ^{13}C NMR ($CDCl_3$) δ: 14.0 (q), 14.1 (q), 22.6 (t), 25.5 (t), 28.2 (t), 29.2 (t), 29.3 (t), 29.4 (t), 29.5 (t), 29.6 (t), 29.7 (t), 29.8 (t), 29.9 (t), 31.8 (t), 32.5 (t), 52.1 (q), 52.2 (d), 54.5 (d), 79.9 (s), 155.6 (s), 171.9 (s), 172.7 (s); IR ($CHCl_3$) (cm^{-1}) 3684, 3435, 2928, 2855, 1737, 1681, 1498, 1466, 1369, 1165, 994, 897, 858; MS *m/z* (relative intensity) 640 $(M + 1)^+$ (1), 582 (1), 565 (1), 386 (5), 326 (6), 270 (92), 226 (100), 88 (7), 57 (83). Anal. Calcd. for $C_{38}H_{74}N_2O_5$: C, 71.43; H, 11.67; N, 4.38. Found: C, 71.20; H, 11.89; N, 4.21.

General Method for Esterification of Carboxylic Acids. Preparation of Methyl (2*S*)-2-*t*-Butoxycarbonylamino-hexadecanoate (18). The N-Boc amino acid **12** (500 mg, 1.35 mmol) was dissolved in dry Et_2O (10 mL) and an ethereal solution of CH_2N_2 was added dropwise until completed evolution of gas. The solution was evaporated and purified by silica gel column chromatography to yield **18** (508 mg, 98% yield) as a solid: mp = 32 °C; $[\alpha]_D^{25}$ +5.8 (*c* 2.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ: 0.87 (t, J = 6.6 Hz, 3H), 1.24 (br s, 24H), 1.44 (s, 9H), 1.60 (m, 1H), 1.75 (m, 1H), 3.72 (s, 3H), 4.27 (d, J = 5.8 Hz, 1H), 4.99 (d, J = 9.2 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ: 14.0 (q), 14.1 (q), 22.6 (t), 25.2 (t), 25.9 (t), 28.0 (t), 28.3 (t), 29.1 (t), 29.2 (t), 29.3 (t), 29.4 (t), 29.5 (t), 29.6 (t), 31.9 (t), 32.7 (t), 52.1 (q), 53.4 (d), 79.7 (s), 155.3 (s), 173.5 (s); IR ($CHCl_3$) (cm^{-1}) 3444, 2928, 2855, 1740, 1711, 1504, 1457, 1361, 1164, 1055, 1006, 858; MS *m/z* (relative intensity) 386 $(M + 1)^+$ (1), 330 (9), 270 (14), 226 (34), 59 (23), 57 (100). Anal. Calcd. for $C_{22}H_{43}NO_4$: C, 68.53; H, 11.24; N, 3.63. Found: C, 68.55; H, 11.41; N, 3.43.

General Method for Reduction of N-Boc Esters. Preparation of (2*S*)-2-*t*-Butoxycarbonylamino-hexadecan-1-ol (19). The N-Boc amino ester **18** (400 mg, 1 mmol) was dissolved in dry benzene (10 mL) at rt. Then DIBAL® was added slowly dropwise (2.2 mL, 1.0 M in hexane, 2.2 mmol). After 5 min to the reaction mixture H_2O (0.3 mL) was added with stirring. The mixture was dried over $MgSO_4$ and filtered through a pad of celite, the solvent was evaporated and the residue was purified by silica gel column chromatography, to yield **19** (325 mg, 90% yield) as a solid: mp = 55 °C; $[\alpha]_D^{25}$ -8.5 (*c* 2.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ: 0.87 (t, J = 5.8 Hz, 3H), 1.25 (br s, 24H), 1.44 (s, 9H), 1.48 (m, 2H), 2.60 (s, 1H, OH), 3.52 (m, 1H), 3.63 (t, J = 10.3 Hz, 2H), 4.65 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ: 14.0 (q), 14.1 (q), 22.6 (t), 26.0 (t), 28.2 (t), 28.3 (t), 28.4 (t), 28.5 (t), 29.3 (t), 29.4 (t), 29.5 (t), 29.6 (t), 29.7 (t), 31.5 (t), 31.8 (t), 52.9 (d), 66.0 (t), 79.5 (s), 156.6 (s); IR ($CHCl_3$) (cm^{-1}) 3691, 3443, 2981, 2928, 2855, 1704, 1711, 1504, 1466, 1368, 1167, 1048, 850; MS *m/z* (relative intensity) 358 $(M + 1)^+$ (5), 302 (26), 270 (18), 226 (53), 59 (15), 57 (100). Anal. Calcd. for $C_{21}H_{43}NO_3$: C, 70.54; H, 12.12; N, 3.92. Found: C, 70.71; H, 12.40; N, 3.78.

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