23. Syntheses of Tetrahydrolipstatin and Absolute Configuration of Tetrahydrolipstatin and Lipstatin

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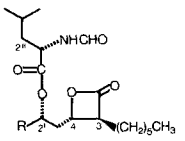
Lipstatin (**1**), a natural product, and tetrahydrolipstatin (**2**) are pancreatic lipase inhibitors. Non-stereoselective and partially stereoselective syntheses of **2** are used to establish the absolute configuration of tetrahydrolipstatin and lipstatin.

Lipstatin (**1**) is a pancreatic lipase inhibitor of microbial origin [1] [2] which, by catalytic hydrogenation, has yielded tetrahydrolipstatin (**2**) [1] [3]. Because of our interest in the biological activity of these compounds, we became interested in the synthesis of this class of β-lactones, and the synthetic intermediates served to establish the absolute configuration of **1** and **2**.



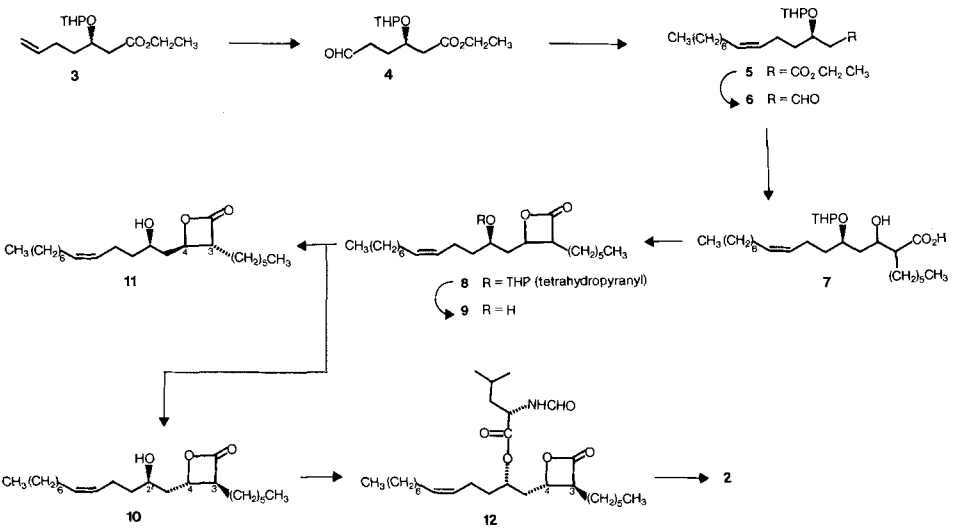
1 R = (*Z,Z*)-$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CHCH_2-$; lipstatin[a])
2 R = $CH_3(CH_2)_{10}-$; tetrahydrolipstatin[a])

[a]) For systematic atom numbering, see *Exper. Part.*

Synthesis of Tetrahydrolipstatin (2) Starting from Ethyl (*R*)-3-Hydroxy-6-heptenoate *(Scheme 1).* – The known ethyl (*R*)-3-hydroxy-6-heptenoate was protected as its tetrahydropyranyl ether **3** [4] which, by ozonolysis, gave aldehyde **4**. *Wittig* reaction yielded ester **5** which was reduced with diisobutylaluminium hydride (DIBAH) to aldehyde **6**. Aldol condensation of the aldehyde with the anion of lithium octanoate gave hydroxy acid **7**. Cyclisation yielded β-lactone **8** as a mixture of diastereoisomers which, by deprotection followed by careful chromatography of the mixture **9**, yielded *trans*-hydroxy-β-lactone **10**[1]) as well as *trans*-hydroxy-β-lactone **11**, the *cis*-isomers being discarded. Esterification of **10** with (*S*)-*N*-formylleucine using *Mitsunobu*'s conditions (inversion of configuration at the OH-substituted center) yielded compound **12** which, by catalytic hydrogenation, gave tetrahydrolipstatin (**2**).

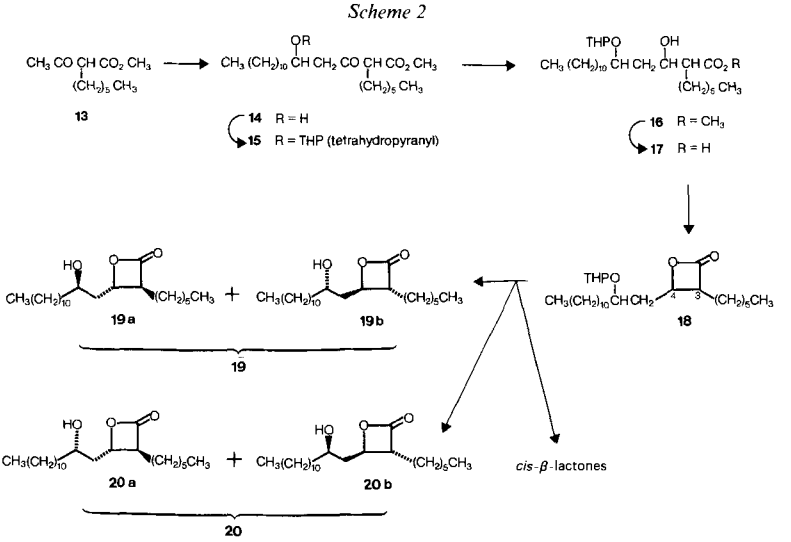
[1]) Absolute configuration as established later in this paper.

Scheme 1

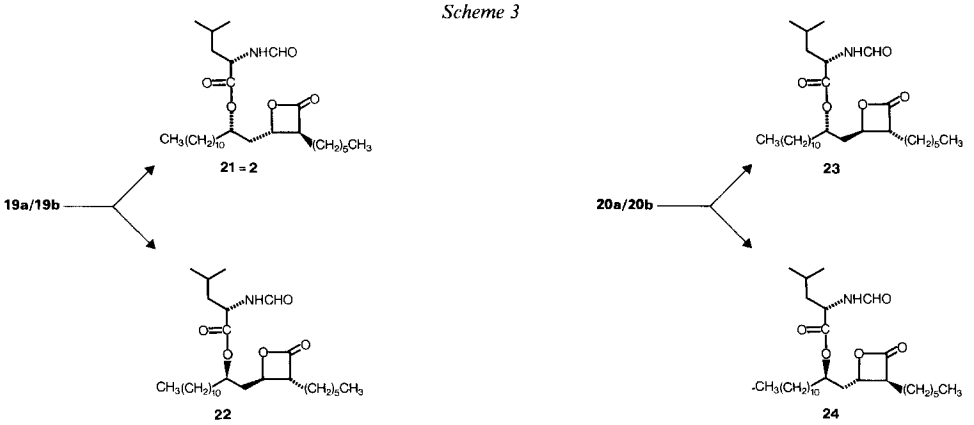


Completely Symmetric Synthesis of Tetrahydrolipstatin (2, *Schemes 2* and *3*). – The second synthesis of **2** starts from the known keto ester **13** which, on condensation with dodecanal, gave compound **14**. Protection of the alcohol function as its tetrahydropyranyl ether **15** and reduction of the keto group gave hydroxy ester **16**. Saponification of **16** and ring closure of the resulting β-hydroxy acid **17** with benzenesulfonyl chloride in pyridine yielded β-lactones **18**. After deprotection of the OH group, the resulting mixture of racemic hydroxy-β-lactones was separated by chromatography into racemic *cis*-β-lactones which were discarded and into racemic *trans*-β-lactones **19** and **20**.

Scheme 2



Scheme 3



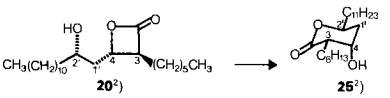
Esterification of **19** with (*S*)-*N*-formylleucine using *Mitsunobu*'s conditions (inversion of configuration of alcohol) yielded two diastereoisomeric esters[1]) **21** and **22**, whilst esterification of **20** under identical conditions gave esters **23** and **24**. One of the four diastereoisomers, namely **21** was identical with tetrahydrolipstatin (**2**) obtained from natural sources.

Absolute Configuration of Tetrahydrolipstatin (2) and Lipstatin (1). – The use of (*S*)-*N*-formylleucine in the two syntheses of tetrahydrolipstatin described above establishes the (*S*)-configuration of the amino-acid part of **1** and **2**. The absolute configuration at C(2′) is (*S*) as established by the synthesis of **2** starting from ethyl (*R*)-3-hydroxy-6-heptenoate, the configuration at the OH-substituted center being inverted in the *Mitsunobu* reaction *(Scheme 1)*. The absolute configuration at these two centers was established independently by *Hochuli et al.* [3] using lipstatin isolated from fermentation broth.

There remains then the establishment of the configuration at C(3) and C(4) on the β-lactone moiety. As the relative configuration at the β-lactone is *trans*, the absolute configuration is either (3*R*,4*R*) or (3*S*,4*S*), and the two possible absolute configurations of **1** and **2** are (2″*S*,2′*S*,3*S*,4*S*) or (2″*S*,2′*S*,3*R*,4*R*). If the relationship between C(2′) and C(4) could be established, the absolute configuration at C(3) and C(4) would be known. In the synthesis described in *Schemes 2* and *3*, the two diastereoisomeric esters **21** (= **2**) and **22** obtained from **19** have the absolute configuration of hydroxy-β-lactones **20** because of the inversion in the esterification step. For this reason, **20** was transformed in analogy to a known literature procedure [5] into the hydroxy-lactone **25** (*Scheme 4*[2])).

NMR analysis of **25** showed the OH function to be axial, the two alkyl groups being equatorial. This establishes the relative configuration of **25** and thus of **20** to be

Scheme 4



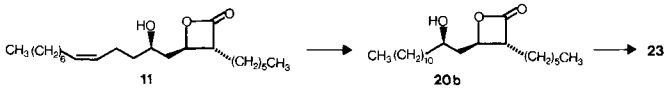
[2]) Only one enantiomer is shown. The atom numbering of **25** corresponds to the one of **1** and **2**.

(2′*R**,3*R**,4*R**). As lipstatin (**1**) and tetrahydrolipstatin (**2**) have been shown to have (*S*)-configuration at C(2′), their absolute configuration is (2″*S*,2′*S*,3*S*,4*S*).

Thus, tetrahydrolipstatin (**2**) is formed from hydroxy-β-lactone **19a.** The enantiomeric hydroxy-β-lactone **19b** must have the absolute configuration (2′*S*,3*R*,4*R*), and compound **22**, diastereoisomeric to **2**, has the absolute configuration (2″*S*,2′*R*,3*R*,4*R*).

As tetrahydrolipstatin (**2**) was previously obtained from hydroxy-β-lactone **10** (see *Scheme 1*), the absolute configuration of the latter is (2′*R*,3*S*,4*S*). Its diastereoisomer **11** is (2′*R*)-configurated, and as it is the only other possible *trans*-β-lactone, **11** has (2′*R*,3*R*,4*R*) configuration. Hydrogenation of **11** yielded hydroxy-β-lactone **20b** which, by esterification under *Mitsunobu*'s condition, gave compound **23**, one of the diastereoisomers of tetrahydrolipstatin (**2**), and its absolute configuration is thus (2″*S*,2′*S*,3*R*,4*R*) (see *Scheme 5*). As **20a** is the enantiomer of **20b**, it has (2′*S*,3*S*,4*S*)- and hence **24** (2″*S*,2′*R*,3*S*,4*S*)-configuration.

Scheme 5



Our thanks are due to Mr. *C. Bardeanu* and *R. Simon* for their excellent technical assistance, colleagues from *Hoffmann-La Roche* Central Research for spectral data and elemental analyses, and Dr. *E. Kupfer* for a sample of tetrahydrolipstatin of natural origin.

Experimental Part

General. Column chromatography: *Merck* silica gel *60* (70–230 mesh ASTM). M.p.: *Tottoli* capillary melting point apparatus; uncorrected. IR ([cm^{-1}]): *Nicolet 7199 FT-IR.* ^{1}H-NMR (δ [ppm] relative to internal TMS; *J* in Hz): *Bruker WM 250.* MS: *MS9-ZAB*, data system *SS200.*

*Ethyl (*R*)-5-Formyl-3-[(tetrahydro-2*H*-pyran-2-yl)oxy]pentanoate* (**4**). A soln. of ethyl (*R*)-3-[(tetrahydro-2*H*-pyran-2-yl)oxy]-6-heptenoate (**3**; 2.56 g, 10 mmol) in AcOEt (40 ml) was treated at −78° with ozone until appearance of a bluish color. The mixture was warmed to r.t., treated with 5% Pd/C (100 mg), and hydrogenated. After completion of H_2 absorption, the mixture was filtered and the filtrate evaporated to yield 2.41 g of crude **4** which was processed without purification.

*Ethyl (*R*)-3-[(Tetrahydro-2*H*-pyran-2-yl)oxy]-6-tetradecenoate* (**5**). To a stirred soln. of octyl(triphenyl)-phosphonium bromide (36.4 g, 80 mmol) in Et_2O (200 ml) and THF (200 ml) was added dropwise 1.6M BuLi in hexane (60 ml, 1.2 equiv.), and the mixture was kept at r.t. for 1 h. After cooling to −40°, a soln. of **4** (10.41 g, 40 mmol) in Et_2O (40 ml) was added dropwise. The mixture was warmed to r.t., stirred for additional 1.5 h, treated with H_2O (160 ml), and extracted 3 times with Et_2O. The combined org. extracts were dried over Na_2SO_4, filtered, and evaporated. Chromatography of the residue (hexane/AcOEt 3:1) yielded 3.3 g (23.5% from **3**) of amorphous **5**. IR: 1737 (ester), 1654 (C=C). ^{1}H-NMR ($CDCl_3$): 5.32 (*m*, H–C(6), H–C(7)); 4.77 (*m*, OCHO); 4.11 (*q*, CH_3CH_2O); 3.92 (br., H–C(3), 1 H of CH_2OCHO); 3.50 (br., 1 H of CH_2OCHO); 2.5 (*m*, 2 H–C(2)); 1.09–2.35 (br. signals, 25 H); 0.89 (*t*, 3 H–C(14)). MS: 252 ($M^{+\cdot}$ − tetrahydropyranol), 85 (tetrahydropyrane$^+$). Anal. calc. for $C_{21}H_{38}O_4$ (354.53): C 71.14, H 10.80; found: C 71.35, H 10.59.

*(*R*)-3-[(Tetrahydro-2*H*-pyran-2-yl)oxy]-6-tetradecenal* (**6**). A soln. of DIBAH (7.8 ml, 1.2M in toluene, 1.1 equiv.) was added dropwise to a stirred soln. of **5** (3 g, 8.5 mmol) in CH_2Cl_2 (25 ml) at −78°. After the addition, the mixture was kept for 1 h at −78°. i-PrOH (22 ml) was added slowly, the mixture warmed to r.t., and H_2O (8.5 ml) added. After stirring for 30 min, the mixture was filtered, the filter washed with CH_2Cl_2, and the filtrate evaporated. Chromatography of the residue (hexane/AcOEt 8:2) yielded 1.8 g (69%) of amorphous **6**. ^{1}H-NMR ($CDCl_3$): 9.70

(*t*, *J* = 1.5, H–C(1)); 5.17–5.35 (*m*, H–C(6), H–C(7)); 4.60 (br. OCHO); 3.21–4.18 (*m*, H–C(3), CH_2OCHO); 2.50 (*m*, 2 H–C(2)); 1.0–2.16 (br. signals, 11 CH_2); 0.76 (*t*, *J* = 4.5, 3 H–C(14)).

*(5*R*)-2-Hexyl-3-hydroxy-5-[(tetrahydro-2*H*-pyran-2-yl)oxy]-8-hexadecenoic Acid* (**7**). A stirred soln. of (i-Pr)$_2$NH (1.28 g, 12.7 mmol) in THF (5.8 ml) was cooled to 0° and 1.6M BuLi in hexane (7.9 ml, 1 equiv.) was added. After 10 min at 0°, the mixture was cooled to −50°, and a soln. of octanoic acid (0.92 g, 6.3 mmol) in THF (8.7 ml) was added. After stirring for 15 min at −50°, the mixture was warmed to r.t. and stirred for 1 h at r.t. The mixture was cooled to −78° and a soln. of **6** (1.8 g, 5.8 mmol) in THF (5.8 ml) was added dropwise. After stirring for 3 h at −78°, the mixture was warmed to r.t., and a sat. aq. NH_4Cl soln. (23 ml) was added. The mixture was extracted 5 times with Et_2O, the combined org. extract washed with a sat. aq. NH_4Cl soln. dried over Na_2SO_4, filtered, and evaporated to yield crude **7** (2 g), which was used without purification.

*3-Hexyl-4-[(*R*)-2-[(tetrahydro-2*H*-pyran-2-yl)oxy]-5-tridecenyl]-2-oxetanone* (**8**). A stirred soln. of **7** (2.6 g, 5.8 mmol) in pyridine (58 ml) was cooled in an ice-bath, and benzenesulfonyl chloride (2 g, 11.6 mmol) was added. The mixture was kept at 0–5° overnight, then treated with H_2O (100 ml), and extracted 5 times with Et_2O. The combined org. extract was dried over Na_2SO_4, filtered, and evaporated and the residue dried under high vacuum to yield crude **8**.

*(3*S*,4*S*)-3-Hexyl-4-[(*R*)-2'-hydroxy-5'-tridecenyl]-2-oxetanone* (**10**) *and (3*R*,4*R*)-3-Hexyl-4-[(*R*)-2'-hydroxy-5'-tridecenyl]-2-oxetanone* (**11**). A soln. of **8** (1.5 g, 5.8 mmol) in EtOH (58 ml) was treated with pyridinium *p*-toluenesulfonate (1.46 g, 2.9 mmol) and heated at 50° for 4 h. The solvent was evaporated and the residue chromatographed (hexane/AcOEt 8:2) to yield 150 mg of **10**. The more polar fraction gave 100 mg of **11**. **10:** IR: 3530 (OH), 1820 (β-lactone). ^{1}H-NMR ($CDCl_3$): 5.32 (*m*, H–C(5'), H–C(6')); 4.5 (*dt*, *J* = 5.5, 8.5, H–C(4)); 3.75 (*m*, H–C(2')), 3.25 (*m*, H–C(3)); 1.10–2.35 (14 CH_2, O*H*); 0.90 (2 CH_3). MS: 334 ($M^{+\cdot}$ − H_2O). Anal. calc. for $C_{22}H_{40}O_3$ (352.55): C 74.95, H 11.44; found: C 75.04, H 11.44.

11: IR: 3570 (OH), 1820 (β-lactone). ^{1}H-NMR ($CDCl_3$): 5.38 (*m*, H–C(5'), H–C(6')); 4.50 (*m*, H–C(4)); 3.75 (*m*, H–C(2')); 3.29 (*m*, H–C(3)); 1.15–2.31 (14 CH_2, O*H*); 0.87 (2 CH_3). MS: 334 ($M^{+\cdot}$ − H_2O). Anal. calc. for $C_{22}H_{40}O_3$ (352.55): C 74.95, H 11.44; found: C 74.42, H 11.70.

*(S,Z)-1'-[((2''*S*,3''*S*)-3''-Hexyl-4''-oxo-2''-oxetanyl)methyl]-4'-dodecenyl (*S*)-*N*-Formylleucinate* (**12**). A soln. of **10** (150 mg, 0.4 mmol), triphenylphosphine (310 mg, 1.2 mmol), and (*S*)-*N*-formylleucine (190 mg, 1.2 mmol) in THF (4 ml) was treated with diethyl azodicarboxylate (210 mg, 1.2 mmol) and kept at r.t. overnight. The solvent was evaporated and the residue chromatographed (toluene/AcOEt 9:1) to yield 80 mg of **12** (50%). IR: 1820 (β-lactone), 1735 (ester), 1690 (amide). ^{1}H-NMR ($CDCl_3$): 8.21 (*s*, NHC*H*O); 5.91 (*d*, *J* = 10.5, NH); 5.42 (*m*, 1 H, CH=CH); 5.32 (*m*, 1 H, CH=CH); 5.05 (*m*, H–C(2)); 4.68 (*m*, H–C(2'')); 4.28 (*m*, H–C(1')); 3.23 (*m*, H–C(3'')); 1.19–2.32 (*m*, 15 CH_2, $(CH_3)_2CH$); 0.98 (*d*, *J* = 7, CH_3–C(4)); 0.97 (*d*, *J* = 7, CH_3–C(4)); 0.88 (*m*, 2 CH_3CH_2). MS: 494 ($M^{+\cdot}$ + H). Anal. calc. for $C_{29}H_{51}NO_5$ (439.72): C 70.55, H 10.41, N 2.84; found: C 70.61, H 10.62, N 2.77.

*(S)-1'-[((2''*S*,3''*S*)-3''-Hexyl-4''-oxo-2''-oxetanyl)methyl]dodecyl (*S*)-N-Formylleucinate (= Tetrahydrolipstatin;* **2**). A soln. of **12** (2.5 mg) in THF (0.1 ml) was treated with 5% Pd/C (1 mg) and hydrogenated. The mixture was filtered and the filtrate evaporated. The residue was chromatographed (toluene/AcOEt 9:1) to yield 1.5 mg of **2**, identical with an authentic sample (TLC, ^{1}H-NMR).

Methyl 2-Hexyl-5-hydroxy-3-oxohexadecanoate (**14**). Methyl 2-hexyl-3-oxobutyrate (**13**; 4 g, 0.019 mol) was slowly added under stirring to a suspension of NaH (1.057 g of 55% oil dispersion, 0.024 mol; washed with hexane) in 150 ml THF. The mixture was stirred for 1 h at r.t. and then cooled to 0°. To the cooled soln. were added 10.4 ml of 1.6N BuLi in hexane (0.9 equiv.), and the resultant yellow soln. of the dianion was stirred at 0° for 15 min. To the cooled soln. was added slowly dodecanal (4.18 g, 0.023 mol) in 20 ml THF. The ice-bath was removed, and the soln. was stirred at r.t. for 1 h and then hydrolysed with 17 ml of aq. 1N HCl (pH 7). The org. solvent was evaporated, the residue extracted with Et_2O, and the extract dried over Na_2SO_4. After evaporation, the crude oil was chromatographed on silica gel (Et_2O/hexane 1:1) to yield 3.5 g (48%) of **14**; m.p. 35°–38°. IR: 3530 (OH); 1730, 1700 (ester, ketone). ^{1}H-NMR ($CDCl_3$): 3.90–4.25 (*m*, H–C(5)); 3.78 (*s*, CH_3O); 3.53 (*t*, *J* = 7.5, H–C(2)); 2.56–2.81 (*m*, 2 H–C(4), –OH); 1.58–2.11 (*m*, 3 H); 1.1–1.58 (br., 27 H); 0.9 (*t*-like, 2 CH_3CH_2). MS: 366 ($M^{+\cdot}$ − H_2O). Anal. calc. for $C_{23}H_{44}O_4$ (384.60): C 71.83, H 11.53; found: C 71.71, H 11.42.

*Methyl 2-Hexyl-3-oxo-5-[(tetrahydro-2*H*-pyran-2-yl)oxy]hexadecanoate* (**15**). A soln. of **14** (4.1 g, 0.01 mol) and dihydro-2*H*-pyran (4.48 g, 0.05 mol) in 50 ml of CH_2Cl_2 was cooled to 0°, and TsOH (20 mg) was added under stirring. After 30 min, the mixture was heated to r.t., and stirring was continued for 1 h. The soln. was then washed with 100 ml of aq. sat. $NaHCO_3$ soln./aq. sat. NaCl soln./H_2O 1:1:2, dried over Na_2SO_4, and evaporated. The residue was then chromatographed (Et_2O/hexane 1:3) to afford 4.4 g of pure **15** (94%) as an oil. IR: 1720, 1750 (ketone, ester). ^{1}H-NMR ($CDCl_3$): 4.50–4.66 (*m*, OCHO); 4.02–4.19 (*t*-like, H–C(5)); 3.70 (*s*, CH_3O); 3.24–3.58

(*m*, CH_2OCHO); 2.31–3.09 (*m*, H–C(2), 2 H–C(4)); 1.03–2.0 (*m*, 36 H); 0.83 (*t*, *J* = 5.5, 2 CH_3CH_2). MS: 383 ($M^{+\cdot}$ – tetrahydropyranyl). Anal. calc. for $C_{28}H_{52}O_5$ (468.72): C 71.75, H 11.18; found: C 71.78, H 11.33.

*Methyl 2-Hexyl-3-hydroxy-5-[(tetrahydro-2*H*-pyran-2-yl)oxy]hexadecanoate* (**16**). To a soln. of **15** (0.68 g, 1.5 mmol) in THF (100 ml) and MeOH (2 ml) was added portionwise $NaBH_4$ (0.465 g, 12 mmol) under stirring and at r.t. After 1 h, the mixture was carefully hydrolysed with aq. 1N HCl (pH 7), and the org. solvent was evaporated. The residue was then extracted with Et_2O. The org. layer was washed with H_2O and brine, dried over Na_2SO_4, and evaporated. The resulting crude oil (0.67 g) was directly used in the next step without further purification.

*2-Hexyl-3-hydroxy-5-[(tetrahydro-2*H*-pyran-2-yl)oxy]hexadecanoic Acid* (**17**). The crude **16** (0.67 g) was refluxed 4 h in 10 ml of aq. 2.5N NaOH/MeOH 1:1. After cooling, the soln. was neutralised with 5 ml of aq. 2.5N HCl. MeOH was evaporated, and the residue was partitioned between EtO_2/H_2O. The org. phase was dried over Na_2SO_4 and evaporated. The resulting crude oil (0.61 g) was directly used in the next step without purification.

*3-Hexyl-4-{2'-[(tetrahydro-2*H*-pyran-2-yl)oxy]tridecyl}-2-oxetanone* (**18**). Crude **17** (0.61 g) was dissolved in 12 ml of dry pyridine and cooled to 0°. Benzenesulfonyl chloride (0.46 g, 2.6 mmol) was slowly added under vigorous stirring. After 15 min, stirring was stopped. The mixture was kept over night between 0 to 5° and then poured into precooled Et_2O/H_2O. The org. phase was separated, dried over Na_2SO_4, and evaporated. The resulting oil was chromatographed (Et_2O/hexane 1:3) to yield 0.26 g of pure **18** (40% based on **15**) as an oil. IR: 1825 (β-lactone). ^{1}H-NMR ($CDCl_3$): 3.0–5.0 (br., CH_2OCHO, H–C(2'), H–C(4), H–C(3)); 1.07–2.10 (*m*, 38 H); 0.9 (*t*-like, 2 CH_3CH_2). MS: 337 ($M^{+\cdot}$ – tetrahydropyranyl). Anal. calc. for $C_{27}H_{50}O_4$ (438.69): C 73.92, H 11.49; found: C 73.68, H 11.77.

3-Hexyl-4-(2'-hydroxytridecyl)-2-oxetanone (**19** and **20**). A soln. of **18** (0.24 g, 0.68 mmol) and 0.014 g (0.28 mmol) of pyridinium *p*-toluenesulfonate in 10 ml EtOH was warmed to 55° with stirring for 4 h. After cooling, EtOH was evaporated (bath temp. < 40°), and the resulting residue was chromatographed (Et_2O/hexane 1:3). The *trans*-racemate **19a**/**19b** eluted first (18 mg, 10%) followed by the *trans*-racemate **20a**/**20b** (35 mg, 18%). Continuing elution afforded 65 mg (33%) of the mixture of the *cis*-β-lactones (33%), easily distinguishable from the *trans*-lactones by NMR [6]. The *cis*-β-lactones were discarded. **19:** M.p. 44.5–46°. IR: 1810 (β-lactone). ^{1}H-NMR ($CDCl_3$): 4.50 (*dt*, *J* = 4.25, 4.25, 8.5, H–C(4)); 3.75–3.90 (*m*, H–C(2')); 3.26 (*dt*, *J* = 8.8, 4.25, H–C(3)); 1.68–2.0 (*m*, 5 H); 1.14–1.62 (*m*, 28 H); 0.88 (*t*-like, 2 CH_3CH_2). MS: 354 ($M^{+\cdot}$). Anal. calc. for $C_{22}H_{42}O_3$ (354.58): C 74.52, H 11.94; found: C 74.58, H 12.16.

20: M.p.: 45.5–47°. IR: 1810 (β-lactone). ^{1}H-NMR ($CDCl_3$): 4.42–4.53 (*m*, H–C(4)); 3.73–3.88 (*m*, H–C(2')); 3.27–3.38 (*m*, H–C(3)); 1.08–2.12 (*m*, 33 H); 0.81–0.95 (*t*-like, 2 CH_3CH_2). Anal. calc. for $C_{22}H_{42}O_3$ (354.58): C 74.52, H 11.94; found: C 74.48, H 12.25.

*1-[(3-Hexyl-4-oxo-2-oxetanyl)methyl]dodecyl (*S*)-*N*-Formylleucinate* (**21–24**). a) *Typical Procedure:* To a soln. of **19** (149 mg, 0.42 mmol), triphenylphosphine (110 mg, 0.42 mmol), and (*S*)-*N*-formylleucine (67 mg, 0.42 mmol) in 1 ml of dry THF at 0°, diethyl azodicarboxylate (78 mg, 0.42 mmol) in 0.5 ml THF was added slowly under stirring. After the addition, the soln. was allowed to warm to r.t., and stirring was continued overnight. The solvent was then evaporated and the residue chromatographed (hexane/$CHCl_3$/dioxane 3:1:0.4). The less polar **21** eluted first (55 mg, 26%) and was identical in all aspects with **2** of natural origin. Continuing elution afforded 89 mg (43%) of **22**.

From **20**, **23** and **24** (more polar than **23**) were obtained.

b) *(*S*)-1'-[((2''*S*,3''*S*)-3''Hexyl-4''-oxo-2''-oxetanyl)methyl]dodecyl (*S*)-*N*-Formylleucinate* (**21**≡**2**): M.p. 41–42.5°. $[\alpha]_D^{20} = -34.45°$ (*c* = 1, $CHCl_3$). IR: 3330 (NH); 1840 (β-Lactone); 1710, 1730 (ester); 1670, 1680 (amide); 1520 (amide II). ^{1}H-NMR ($CDCl_3$): 8.23 (*s*, NHC*H*O); 5.93 (*d*, *J* = 8, NH); 5.03 (*m*, H–C(2)); 4.68 (*dt*, *J* = 8.5, 8.5, 4.5, H–C(2'')); 4.25–4.34 (*m*, H–C(1')); 3.22 (*dt*, *J* = 8.3, 8.3, 4.5, H–C(3'')); 1.91–2.25 (*m*, CH_2–C(2'')); 1.25–1.84 (*m*, 33 H); 0.96 (*d*, *J* = 5.3, $(CH_3)_2CH$); 0.84–0.94 (*t*-like, 2 CH_3CH_2). MS: 337 ($M^{+\cdot}$ – $(CH_3)_2CHCH_2CH(NCHO)COO^{\cdot}$). Anal. calc. for $C_{29}H_{53}NO_5$ (495.75): C 70.26, H 10.78, N 2.89; found: C 70.04, H 10.76, N 2.84.

c) *(*R*)-1'-[((2''*R*,3''*R*)-3''-Hexyl-4''-oxo-2''-oxetanyl)methyl]dodecyl (*S*)-*N*-Formylleucinate* (**22**): Amorphous. $[\alpha]_D^{20} = -2.2°$ (*c* = 0.9, MeOH). ^{1}H-NMR ($CDCl_3$): 8.21 (*s*, NHC*H*O); 5.88 (*d*, *J* = 8, NH); 4.96–5.09 (*m*, H–C(2)); 4.67 (*dt*, *J* = 4, 7.5, 7.5, H–C(2'')); 4.25–4.36 (*m*, H–C(1')); 3.22 (*dt*, *J* = 7.5, 7.5, 4, H–C(3'')); 1.97–2.26 (*m*, CH_2–C(2'')); 1.03–1.5 (*m*, 33 H); 0.94–1.03 (4*m*, $(CH_3)_2CH$); 0.75–0.94 (*m*, 2 CH_3).

d) *(*S*)-1'-[((2''*R*,3''*R*)-3''-Hexyl-4''-oxo-2''-oxetanyl)methyl]dodecyl (*S*)-*N*-Formylleucinate* (**23**): Amorphous. $[\alpha]_D^{20} = -2.87°$ (*c* = 0.8, MeOH). ^{1}H-NMR ($CDCl_3$): 8.25 (*s*, NHC*H*O); 5.97 (*d*, *J* = 8, N*H*CHO); 4.94–5.06 (*quint.*-like, H–C(2)); 4.71 (*dt*, *J* = 5, 9, 9, H–C(2'')); 4.20–4.33 (*m*, H–C(1')); 3.20–3.29 (*m*, H–C(3'')); 2.00–2.09 (*t*-like, CH_2–C(2'')); 1.04–1.87 (*m*, 33 H); 0.93–1.03 (*m*, $(CH_3)_2CH$); 0.78–0.93 (*m*, 2 CH_3CH_2).

e) *(*R*)-1'-[((2''*S*,3''*S*)-3''-Hexyl-4''-oxo-2''-oxetanyl)methyl]dodecyl (*S*)-*N*-Formylleucinate* (**24**): Amorphous. $[\alpha]_D^{20} = -19.4°$ (*c* = 0.35, MeOH). ^{1}H-NMR ($CDCl_3$): 8.21 (*s*, NHC*H*O); 5.93 (*d*, *J* = 8.5, N*H*CHO);

5.0–5.12 (*quint.*-like, H–C(2)); 4.68 (*dt*, J = 5, 8.5, 8.5, H–C(2″)); 4.25–4.36 (*m*, H–C(1′)); 3.23 (*dt*, J = 5, 8, 8, H–C(3″)); 2.01–2.12 (*m*, CH_2–C(2″)); 1.06–1.9 (*m*, 33 H); 0.93–1.06 (*m*, $(CH_3)_2CH$); 0.77–0.93 (*m*, 2 CH_3CH_2).

*3α-Hexyl-3,4,5,6-tetrahydro-4α-hydroxy-6β-undecyl-2*H*-pyran-2-one* (**25**). For 4 h, **20** (100 mg, 0.28 mmol) was heated under reflux in a mixture of 0.56 ml of aq. 1N KOH (0.56 mmol) and 35 ml of dioxane. After cooling to r.t., the soln. was neutralised with 0.56 ml of 1N aq. HCl and then evaporated. The residue was partitioned between Et_2O and H_2O and the org. layer dried over Na_2SO_4. After evaporation of the Et_2O, the residue was dissolved in CH_2Cl_2 (30 ml), treated with a cat. amount of TsOH for 2 h at r.t., and then washed with sat. aq. $NaHCO_3$ soln./sat. aq. NaCl soln./H_2O 1:1:2. The org. layer was dried over Na_2SO_4 and evaporated, and the residue was recrystallised from Et_2O/hexane to yield 60 mg (60%) of pure **25**. M.p. 108–109°. IR: 3400 (OH); 1690 (OCO). ^{1}H-NMR ($CDCl_3$): 4.64–4.78 (br. *m*, H–C(6)); 4.27–4.34 (br. *s*, H_{eq}–C(4)); 2.25–2.33 (*m*, 1 H); 2.03–2.19 (*m*, 2 H): 1.91 (br. *s*, OH); 1.17–1.77 (*m*, 30 H); 0.88 (*t*-like, 2 CH_3CH_2). MS: 354 ($M^{+\cdot}$). Anal. calc. for $C_{22}H_{42}O_3$ (354.28): C 74.52, H 11.94; found: C 74.51, H 12.00

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