120. Tetrahydrolipstatin: Degradation Products Produced by Human Carboxyl-ester Lipase

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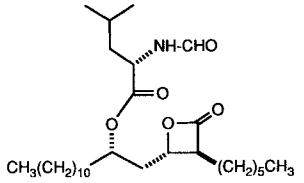
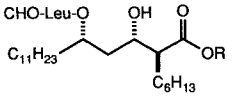
The fate of tetrahydrolipstatin (**1**) incubated with human carboxyl-ester lipase (HCEL) was investigated. The primary metabolite was identified as δ-(*N*-formyl-L-leucyloxy)-β-hydroxy acid **2a** with conserved configuration. It is formed by attack of the active-site serine of HCEL at the carbonyl C-atom of the β-lactone ring of **1** followed by hydrolysis of the intermediate serine ester. Further products isolated were identified and a complete degradation scheme is proposed.

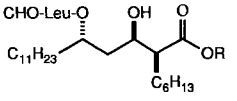
1. Introduction. – Tetrahydrolipstatin (THL = (*S*)-1-{[(2*S*,3*S*)-3-hexyl-4-oxooxetan-2-yl]methyl}dodecyl *N*-formyl-L-leucinate; US-adopted name: 'orlistat'; **1**) is a specific inhibitor of triacylglycerol lipases, especially of pancreatic lipase, and at present is in phase III of clinical development as an antiobesity agent [1–3]. It forms a covalent bond with the active-site serine of pancreatic lipase (PL) from pig and man [4] [5]. Other serine hydrolases such as gastric lipase and pancreatic carboxyl-ester lipase[1]) (CEL) are also inhibited by **1**, and CEL was shown to undergo temporary inhibition [2]. This



1 tetrahydrolipstatin



2a R = H
b R = Me



3a R = H
b R = Me

CHO-Leu = *N*-formyl-L-leucyl

property prompted us to attempt the isolation and structural elucidation of the metabolite(s) formed from **1** incubated with human CEL (HCEL). We report herein the identification of the primary degradation products of **1** formed with HCEL and propose a pathway for the formation of the various products.

[1]) Synonyma: cholesterol esterase, steryl-ester hydrolase, carboxyl-ester hydrolase, carboxyl esterase, non-specific lipase [6].

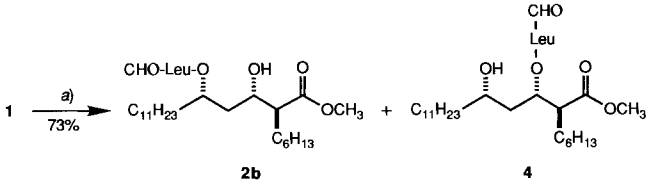
2. Results. – 2.1. *General.* The β-lactone moiety being the most reactive part in tetrahydrolipstatin (**1**), we anticipated products derived from **1** by either attack of a nucleophile, *e.g.* the OH group of the active-site serine, at the carbonyl C-atom or at C(β) of the β-lactone ring. This expectation is supported by the adducts of **1** formed irreversibly with porcine and human PL [4] [5]. With both lipases, Ser^{152} attacks the β-lactone ring exclusively at the carbonyl C-atom forming a serine ester. Products occurring by nucleophilic attack at C(β) (formation of a serine ether), as observed, *e.g.*, with H_2O [7], were not found. Therefore, in a reversible system like HCEL incubated with **1** in which **1** is consumed by the enzyme, we expected, after recovery of HCEL activity, to find β-hydroxy acid **2a** as product of hydrolysis. The formation of its 3-epimer **3a**, the product of an attack of active site serine at C(β), was expected, if at all, in trace amounts.

As hydroxy acids **2a** and **3a** are reactive and form more stable compounds by isomerization and lactonization, they have to be quenched, *e.g.* as esters **2b** and **3b**, respectively, if the primary metabolite(s) are to be identified [7]. The resulting esters are accessible to GC/MS and HPLC analysis [7]. Tests with a mixture of **1** and degradation products thereof showed that the hydroxy acids formed are stabilized in the mixture [7] and rearrange at room temperature only after weeks to the corresponding δ-lactones, and that the hydroxy acids of the mixture in $MeOH/Et_2O$ give the corresponding methyl esters in nearly quantitative yield on addition of diazomethane in Et_2O.

To elucidate the configuration of the degradation products, the relative HPLC retention time(s) (t_R) of the isolated diazomethane-quenched metabolite(s) of **1** were compared with that of the independently synthesized stable methyl β-hydroxycarboxylates **2b** and **3b**. Products with inverted configuration at C(α) or C(δ) were not anticipated because under harsher conditions than those used for the workup of the incubation with HCEL (*cf. Exper. Part*), we never observed compounds arising from epimerization at one or both these centers [7].

2.2. *Syntheses.* Treatment of a tetrahydrolipstatin (**1**) solution in MeOH with a catalytic amount of $Et_3N/AcOH$ 1.3:1 provided a mixture of the regioisomeric hydroxy esters **2b** and **4** (*Scheme 1*). Their ratio (78:22) was independent of the workup conditions and also was not influenced by changing the catalyst or by using mixtures of MeOH with other solvents. Separation of **2b**/**4** was achieved by prep. HPLC (*RP-8* column). Both isomers **2b** and **4** proved to be stable below room temperature for months; in solution ($MeCN/H_2O$ 6:4, MeOH, or MeCN) at room temperature, the isomers were fairly stable, and equilibrium by 1,3-shift of the amino-acid side chain was not attained within a month.

Scheme 1

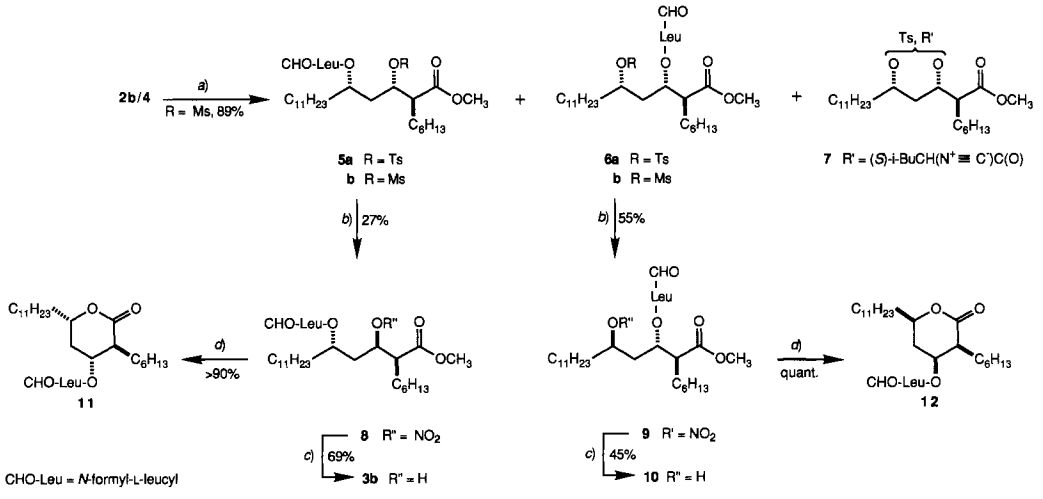


CHO-Leu = *N*-formyl-L-leucyl

a) MeOH, $Et_3N/AcOH$ 1.3:1, 50°, 3 d; prep. HPLC on *RP-8* column, $MeCN/H_2O$ 6:4.

For the synthesis of the 3-epimer **3b**, we initially followed the protocol developed by *Seebach* and coworkers for the epimerization of ethyl 3-hydroxybutyrate [8]. Tosylation of the regioisomer mixture **2b**/**4** provided *N*-(formylamino)-tosylates **5a**/**6a** as the main products together with isocyano-tosylates **7**, by-products which were formed by dehydration of the desired tosylates **5a**/**6a** (*Scheme 2*). The separation of **5a**/**6a** by repeated flash

Scheme 2



a) $MeSO_2Cl$, *Hünig*'s base, cat. 4-$(Me_2N)C_5H_4N$, pentane, 0–5°; then r.t., 5 h; further add. of $MeSO_2Cl$ and *Hünig*'s base, r.t., 20 h. b) $(Bu_4N)NO_3$, toluene, reflux, 3 h. c) H_2 (10 bar), 10% Pd/C, i-PrOH, r.t., 90 min. d) TsOH (cat.), MeCN, r.t., 2 d.

chromatography (silica gel) proved to be difficult, but furnished 3-tosyloxy ester **5a** as the main product together with impure 5-tosyloxy regioisomer **6a**. Subsequently, we found that the formation of the isocyano-tosylates **7** was nearly completely suppressed by preparing the mesylates **5b**/**6b**, which were obtained under far milder conditions than the bulkier tosylates. Furthermore, the mesylates **5b**/**6b** were easy to separate giving the regioisomers in fair yield[2]). S_N2 Substitution of the nucleofugal groups in **5b** or **6b** with nitrate ions following the protocol of *Cainelli et al.* furnished nitrate esters **8** and **9**, respectively [9]. Finally, hydrogenolysis of the nitrates yielded (3*R*)- and (5*R*)-hydroxy ester **3b** and **10**.

The absolute configuration of **3b** and **10** was checked by NMR analysis of the corresponding δ-lactones which were obtained from the acylic precursors by acid-catalyzed isomerization (1,3-acyl shift in **3b**) and transesterification. β-Hydroxy ester **3b** furnished the known (3*S*,4*R*,6*S*)-lactone **11** [7], whereas δ-hydroxy isomer **10** provided

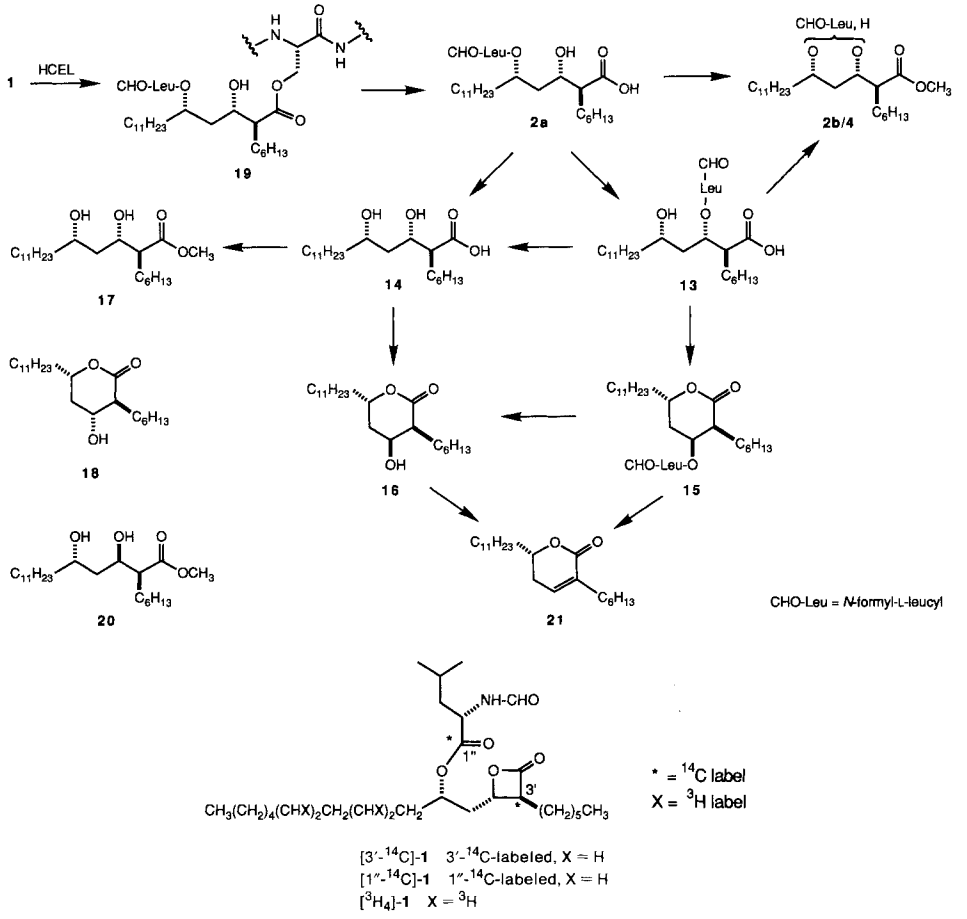
[2]) Attempts to prepare the triflates **5**/**6** (R = CF_3SO_2) with $(CF_3SO_2)_2O$/pyridine or CF_3SO_2Cl/Et_3N/4-$(Me_2N)C_5H_4N$ as acylating agents failed: an inseparable mixture of either chlorinated products (substitution of the triflate group by Cl with CF_3SO_2Cl as reagent) or products derived from starting material by reaction of both acylating agents with both the OH group and the *N*-formylleucyl moiety was obtained.

the new δ-lactone **12** with (3*S*,4*S*,6*R*)-configuration. The latter was independently prepared from (3*S*,4*S*,6*R*)-3-hexyltetrahydro-4-hydroxy-6-undecyl-2*H*-pyran-2-one [10] by esterification with *N*-formyl-L-leucine.

In contrast to the *N*-formylleucine derivatives **2b** and **4** (*Scheme 1*) with conserved configuration, none of the isomers **3b** and **10** (*Scheme 2*), in various solvents at temperatures up to 50°, showed 1,3-shift of the amino-acid side chain. Therefore, we conclude that, under the workup conditions used for the isolation of the degradation products of **1** formed with HCEL, no products with inverted configuration at C(β) or C(δ) were formed and no side-chain migration occurred in such compounds.

2.3. *Incubations.* 2.3.1. *General.* The incubations with HCEL were performed with ^{14}C-labeled tetrahydrolipstatin (**1**), mostly with the α-^{14}C-labeled [3′-^{14}C] **1** (*cf. Scheme 3*), and the consumption of **1** traced by TLC. After workup, the degradation products were esterified with diazomethane in Et_2O for HPLC (UV and radio detection) and subsequently silylated for GC/MS analysis.

Scheme 3



2.3.2. *TLC Analyses.* The incubation experiments of **1** with HCEL as well as the blank experiments run without enzyme were routinely traced by TLC. In *Table 1*, a typical time course of a **1**/HCEL incubation with freshly purified HCEL is shown. Obviously, **1** was consumed by the enzyme in a time-dependent manner, and after 48 h, less than 3% of **1** remained unchanged. In the control experiment without enzyme, still more than 84% of **1** was detectable after the same period of time. During the workup procedure, part of the remaining **1** was further degraded leading to *ca.* 99% turnover in **1** for the incubation and to *ca.* 22% for the blank experiment. For this further degradation, an enzymatically catalyzed reaction could be excluded, because the enzyme was inactivated by the lyophilization and extraction, the first steps of the workup protocol (*cf. Exper. Part*).

The main products were very polar (located near the starting spot), and < 2% of the degradation products were less polar than **1**. Comparison with reference compounds showed that the products in *Zone 1* were probably (*N*-formylleucyloxy)-hydroxy acids, *i.e.* **2a** and/or **13**, or dihydroxy acids, *i.e.* **14**, and those in *Zones 2* and *4* δ-lactones, *i.e.* **15** and **16** (see *Scheme 3*).

Table 1. *Partition of ^{14}C Activity* (in %) *as Shown by TLC for Aliquots Taken from the Incubation of [3′-^{14}C]Tetrahydrolipstatin ([3′-^{14}C]-**1**) with HCEL or from the Blank Experiment*

TLC *Zone*	0 h		1 h		6 h		24 h		48 h		After workup	
	inc.	blank	inc.	blank	inc.	blank	inc.	blank	inc.	blank	inc.	blank
5 (front)	–	–	–	–	–	–	–	–	–	–	–	–
4	–	–	–	–	–	–	0.6	–	1.6	0.7	6.0	0.4
3 ([3′-^{14}C]-**1**)	98.8	98.8	93.5	98.7	60.5	96.7	5.1	94.0	2.5	84.0	1.2	77.6
2	–	–	–	–	–	1.2	–	–	1.0	1.0	1.1	11.4
1 (start)	1.1	1.1	6.5	1.3	39.5	2.0	94.3	6.0	95.0	14.3	91.7	10.6

Prior to workup, nearly no δ-lactone(s) were found after 24 h in both the incubation and the control experiment. After workup, the part of δ-lactones was nearly tripled, from 2.6 to 7.1%, for the incubation and even more increased, *i.e.* from 1.7 to 11.8%, for the control experiment. This indicates that these products were artifacts produced either from **1** (especially in the blank experiment) or, more important for the incubation, from

Table 2. *$^{3}H/^{14}C$ Ratio in the Upper and Lower Phase after Partition of Aliquots Taken from the Incubation of [$^{3}H_4$]-**1**/[1″-^{14}C]-**1** with HCEL or from the Blank Experiment at Various Times.* Activities in dpm.

t [h]	Incubation					Blank				
	upper phase		lower phase		ratio $^{3}H/^{14}C$[a])	upper phase		lower phase		ratio $^{3}H/^{14}C$[a])
	^{3}H	^{14}C	^{3}H	^{14}C		^{3}H	^{14}C	^{3}H	^{14}C	
1	–	–	985	336	1.00	–	–	1220	413	1.00
6	–	–	1043	346	1.03	–	–	1569	495	1.07
24	–	–	1365	442	1.05	–	–	1625	552	1.00
48[b])	–	–	993	330	1.03	–	–	1078	351	1.04
lyophilized	99	73	10308	3370	1.04	169	177	11656	4073	0.97
extracted	1238	710	21029	7202	1.00	313	380	15738	5277	1.01

[a]) Normalized for t = 1 h: 1.00! b) *Ca.* 80% turnover.

the polar and reactive hydroxy acids found in *Zone 1* of the TLC (*cf. Chapt. 3*). Furthermore, with high turnover and prolonged reaction time, the percentage of δ-lactones (*Zones 2* and *4*) increased considerably (*cf.* values for 24 *vs.* 48 h in *Table 1*). α,β-Unsaturated δ-lactone **21**, the final product of the degradation cascade [7] (detected by TLC in some incubations in *Zone 5*; < 1% of total activity after workup and in larger, variable amount after transformation to the derivatives), was found in amounts of less than 5%.

The efficiency of our workup procedure was validated with doubly labeled **1**, *i.e.* [$^3H_4$]-**1**/[1″-^{14}C]-**1**, which was submitted to the usual experimental conditions. This showed that for both the incubation and blank experiment, **1** and derivatives thereof were extracted into the organic layer, and that the initial $^3H/^{14}C$ ratio was maintained throughout all steps (*Table 2*; *cf. Chapt. 3*). By extraction, *ca.* 70% of ^3H and/or ^{14}C activity was recovered. The missing 30% was lost with the unhydrolyzed **1**/HCEL adduct.

2.3.3. *GC/MS Analyses.* Beside recovered **1**, we identified by GC/MS two kinds of β-lactone-cleavage products, one corresponding to **2b** and to another regio- or stereoisomer still bearing the amino-acid side chain, the other corresponding to dihydroxy ester **17** (configurations undetermined; see *Scheme 3*). Furthermore, we found in low amount a 4-hydroxy-δ-lactone corresponding to **16**, together with traces of a *N*-formylleucyl ester thereof, *i.e.* corresponding to **15** (configurations undetermined).

2.3.4. *HPLC Analyses.* The configurations of the products derived from **1** were investigated using HPLC by comparing the relative retention time (t_R) found for the esterified degradation products with those of reference compounds (*cf. Table 3*). The results revealed that in all identified compounds the initial configuration of **1** was conserved, *i.e.* for the leucine derivative, structure **2b**, and for the dihydroxy compound, structure **17** were confirmed (*cf. Scheme 3*). The δ-lactones were identified as **15** and **16** with (all-*S*)-configuration. Compound **16** was found in variable amounts in all analyzed incubations but represented at best 10% of the labeled products. The 4-hydroxy epimer **18** of **16** (*Scheme 3*) and its *N*-formylleucine derivative **11** (*Scheme 2*) were not detected.

Table 3. *GC/MS and HPLC Analyses of the Incubation Mixtures*

	t_R[a])	Comment		t_R[a])	Comment
1	*1.00*	60–99% consumed by HCEL	**15**	0.81	traces (GC/MS; HPLC (?))
2b	0.80	with *ca.* 60% turnover: main product	**16**	0.68	identified by HPLC and GC/MS
3b	0.91	not found	**17**	0.84	with > 90% turnover: main product
4	0.90	identified by HPLC only; *cf. Chapt. 3*	**18**	0.61	not found
10	1.05	not found	**20**	0.63	not found
11	0.86	not found	**21**	1.50	in variable amounts
12	0.76	not found			(artifact, *cf. Chapt. 2.3.2*)

[a]) HPLC Retention times relative to that of **1**.

3. Discussion. – The detection of β-hydroxy ester **2b** in the degradation mixture by GC/MS and HPLC prompted us to search for its regioisomer **4**. With radio detection, we were able to identify this compound in a ratio for **2b** and **4** of *ca.* 4:1. With UV detection alone, it was not possible to identify **4** positively due to superposition with other peaks generated by compounds bearing no radioactive label. The presence of 3-epimer **3b** could not be excluded entirely by HPLC analysis due to its very similar t_R value of 0.91

compared with t_R 0.90 for **4**. But the ratio of *ca.* 4:1 observed for **2b** and **4** in the degradation mixture was in excellent accordance with the ratio of 79 ± 2:21 ± 2 (calculated from 10 independent experiments) for these regioisomers prepared directly from **1** by catalyzed methanolysis (*Scheme 1*). Therefore, we conclude that at best, only traces of **3b** could be hidden under the very small peak of **4** observed by HPLC analysis.

Surprisingly, we observed **2b** and **4** as main products only in incubations with *ca.* 60–80% turnover. With high turnover in **1** (> 90%), the β,δ-dihydroxy ester **17** and 4-hydroxy-δ-lactone **16** became the main products. It is very unlikely that these compounds were generated from **1** directly, because blank experiments led to > 80% recovery of **1**, indicating that the ester bond to the side chain was fairly stable under the incubation and workup conditions. Nevertheless, the formation of dihydroxy ester **17**, especially in incubations with high turnover in **1**, was suspected to be a secondary reaction, *e.g.* a chemically induced, uncatalyzed cleavage of the amino-acid ester link. Moreover, it is well known that amino-acid esters are very poor substrates for lipases [11]. For **1**, this was further corroborated by the incubation with [$^3H_4$]-**1**/[1″-^{14}C]-**1**. The partition of the labels in the products (see *Table 2*) and especially the preserved $^3H/^{14}C$ ratio throughout the complete protocol proves that the *N*-formylleucine group was not cleaved off (tests with the very polar *N*-formylleucine showed that it would be found in the aqueous phase). Only after workup, *i.e.* during storage, formation of derivatives, and analysis, this ratio decreased (GC/MS and HPLC evidence), indicating that the loss of the side chain is an artifact. In incubations with high turnover, this chemically induced cleavage started prior to workup (data not shown) which, finally, led to formation of β,δ-dihydroxy acid **14** as the main product, at the cost of both *N*-formylleucine derivatives **2a** and **13**.

Hence, for the fate of **1** incubated with HCEL, we propose the degradation pathway displayed in *Scheme 3*. In a first step, the active-site serine of HCEL attacks **1** at the carbonyl C-atom of the β-lactone ring, yielding an acylated HCEL derivative **19**. Hydrolysis of this serine ester provides β-hydroxy carboxylic acid **2a** as the primary product which is isomerized either to **13** or loses its amino-acid side chain by hydrolysis to give dihydroxy acid **14**. Ring closure of **14** furnishes 4-hydroxy-δ-lactone **16**, a compound also accessible *via* β-leucyloxy derivative **13** which, after cyclization to β-leucyloxy-δ-lactone **15** and hydrolysis provides **16**.

The alternative nucleophilic attack of the active-site serine at C(β) of **1** would give, *via* a serine ether and after cleavage of the ether bond, hydroxy acid **3a** with inverted configuration at C(3). Formation and possible pathways for cleavage of such serine ethers were discussed by *Lüthi-Peng et al.* for a VIGHS-1 ether adduct [5]. The missing 4-epimers **11** (*Scheme 2*) and **18** of the two δ-lactones **15** and **16** (*Scheme 3*) as well as the absence of both the monohydroxy ester **3b** (*Scheme 2*) and the corresponding dihydroxy ester **20** (*Scheme 3*) in our experiments suggest a nearly quantitative cleavage of the β-lactone ring in **1** by a nucleophilic attack of active-site serine in HCEL at the carbonyl C-atom[3]).

4. Conclusion. – Tetrahydrolipstatin (**1**) incubated with HCEL is attacked by the active-site serine of HCEL at the carbonyl C-atom of the β-lactone ring furnishing a HCEL-**1** serine ester **19** (the inhibitory step) which after hydrolysis (giving full recovery of HCEL activity [2]) yields β-hydroxy acid **2a** still bearing the amino-acid side chain. The

[3]) For a more extended discussion of the nucleophilic cleavage of the β-lactone moiety of **1** by attack of H_2O, *cf.* [7] and ref. cit. therein.

primary degradation product **2a** is quenched as methyl ester **2b** or its regioisomer **4**, the product of a 1,3-acyl shift of the *N*-formylleucine side chain. The other degradation products are all secondary products formed from **2a** by rearrangement (*i.e.* **13**) and hydrolysis (*i.e.* **14**), followed by ring closure (**15** and **16**) and elimination (*i.e.* **21**). All identified products (> 99%) retain the (all-*S*)-configuration of **1**. This indicates that attack at the carbonyl C-atom of **1** is the sole mode of β-lactone cleavage.

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Experimental Part

General. See also [7]. HPLC: *LiChrospher®-100-RP-8 column* (125 × 4 mm, 5 µm; *Merck*); MeCN/H_2O 8:2 (HPLC quality; *Merck*); flow 1 ml/min for 20 min; UV detection (195 nm) with *L4000* detector (*Merck-Hitachi*); injected amount, 20 µg in 20 µl; integration with *D-2500 integrator (Merck-Hitachi)*, values in area-%; t_R = retention times rel. to that of **1** (see *Table 3*).

(S)-1-{[(2S,3S)-3-Hexyl-4-oxo[3-^{14}C]oxetan-2-yl]methyl}dodecyl N-*Formyl*-L-*leucinate* ([3'-^{14}C]-**1**) was prepared with [2-^{14}C]octanoic acid as ^{14}C-source following the procedure of *Barbier et al.* [12] [13].

(S)-1-{[(2S,3S)-3-Hexyl-4-oxooxetan-2-yl]methyl}dodecyl N-*Formyl*-L-*[1-^{14}C]leucinate* ([1''-^{14}C]-**1**) was synthesized by a *Mitsunobu*-type reaction of (3*S*,4*S*)-3-hexyl-4-[(*R*)-2-hydroxytridecyl]oxetan-2-one [10] with *N*-formyl-L-[1-^{14}C]leucine [14].

(S)-1-{[(2S,3S)-3-Hexyl-4-oxooxetan-2-yl]methyl}[3,4,6,7-$^3H_4$]dodecyl N-*Formyl*-L-*leucinate* ([$^3H_4$]-**1**) was prepared by catalytic hydrogenation of lipstatin [15] with tritium gas [14].

1. *Syntheses.* 1.1. *Methyl (2S,3S,5S)-5-[(S)-2-Formamido-4-methylpentanoyloxy]-2-hexyl-3-hydroxyhexadecanoate* (**2b**) *and Methyl (2S,3S,5S)-3-[(S)-2-Formamido-4-methylpentanoyloxy]-2-hexyl-5-hydroxyhexadecanoate* (**4**). To tetrahydrolipstatin (**1**; 0.99 g, 2 mmol) in abs. MeOH (10 ml) was added a soln. of Et_3N (0.82 ml, 5.9 mmol) and AcOH (0.26 ml, 4.55 mmol) in abs. MeOH (10 ml). The mixture was stirred at 50° for 4 d, then the volume reduced at r.t. to *ca.* 5%, the residue taken up in hexane and washed with sat. aq. $NaHCO_3$ soln. and brine, and the org. phase dried and evaporated at r.t. FC (silica gel, hexane/AcOEt 1:1) yielded 0.88 g (84%) of **2b**/**4** 79:21 (HPLC). Colorless oil. IR: 3359 (OH), 1738 (ester), 1669, 1524 (amide). MS: 528 (2, $[M + H]^+$), 510 (9, $M + H - H_2O]^+$). Anal. calc. for $C_{30}H_{57}NO_6$ (527.79): C 68.27, H 10.89, N 2.65; found: C 68.04, H 10.96, N 2.60.

A sample of **2b**/**4** was separated by prep. HPLC (25 mg of **2b**/**4** in 250 µl of MeCN per run; *LiChrosorb® RP-8* column (250 × 10 mm, 7 µm, *Merck*); MeCN/H_2O 6:4, 5 ml/min). From the fractions of interest, MeCN was evaporated at r.t., the residue extracted with Et_2O, and the org. phase washed with brine, dried, and evaporated at r.t.: **2b** in 98% purity and **4** enriched to 67%.

Data of **2b**: IR: 3342 (OH), 1737 (ester), 1669, 1527 (amide). ^{1}H-NMR ($CDCl_3$): 0.88 (*t*-like, J = 7, 2 $MeCH_2$); 0.94, 0.96 (*d*, J = 6, Me_2CH); 1.10–1.40 (*m*, 12 CH_2, Me_2CHCH_2); 1.45–1.85 (*m*, 3 CH_2, Me_2CHCH_2, CH_2(4)); 2.47 (*dt*, J = 5, 8, H–C(2)); 2.78 (*d*, J = 8, OH); 3.72 (*s*, MeO); 3.69–3.82 (*m*, H–C(3)); 4.70 (*dt*, J = 4, 8, C(O)CHN); 5.09 (*quint.*, J = 6, H–C(5)); 6.05 (*d*, J = 8, NH); 8.22 (*s*, CHO).

Data of **4**: ^{1}H-NMR ($CDCl_3$): 0.88 (*t*-like, J = 7, 2 $MeCH_2$); 0.95, 0.96 (*d*, J = 5, Me_2CH); 1.15–1.96 (*m*, 16 CH_2, Me_2CHCH_2); 2.66–2.79 (*m*, H–C(2)); 3.5 (br., OH); 3.70 (*s*, MeO); 3.77–3.90 (*m*, H–C(5)); 4.55–4.72 (*m*, C(O)CHN); 5.21–5.36 (*m*, H–C(3)); 7.37 (*d*, J = 8, NH); 8.29 (*s*, CHO).

1.2. *Methyl (2S,3S,5S)-5-[(S)-2-Formamido-4-methylpentanoyloxy]-2-hexyl-3-[(4-tolyl)sulfonyloxy]-hexadecanoate* (**5a**), *Methyl (2S,3S,5S)-3-[(S)-2-Formamido-4-methylpentanoyloxy]-2-hexyl-5-[(4-tolyl)-sulfonyloxy]hexadecanoate* (**6a**), *and Methyl (2S,3S,5S)-2-Hexyl-3(5)-[(S)-2-isocyano-4-methylpentanoyloxy]-5(3)-[(4-tolyl)sulfonyloxy]hexadecanonate* (**7**). To a chilled soln. of **2b**/**4** (1.20 g, 2.27 mmol) in CH_2Cl_2 (20 ml) were added 4-(dimethylamino)pyridine (0.55 g, 4.54 mmol) and TsCl (0.48 g, 2.50 mmol). The resulting soln. was stirred at r.t. for 3 d, then further 4-(dimethylamino)pyridine (0.27 g, 2.27 mmol) and TsCl (0.24 g, 1.25 mmol) were added and stirred for additional 24 h. Then the mixture was diluted with CH_2Cl_2 and washed twice with buffer soln. of pH 3 and brine, the neutral org. phase dried and evaporated, and the resulting sticky oil purified by FC (silica

gel, hexane/AcOEt 1:1): 1.45 g (94%) of **5a**/**6a** and 0.04 g (3%) of **7**[4]). Repetitive FC (silica gel, CH_2Cl_2/hexane 95:5) of **5a**/**6a** (1.45 g) gave 1.16 g (80%) of **5a** and 0.19 g (13%) of **6a**.

Data of **5a**/**6a**: IR: 3376 (NH), 1742 (ester), 1691, 1520 (amide), 1597, 1495, 815 (aryl), 1368, 1176 (SO_2-aryl). MS: no $M^{+\cdot}$, 523 (0.1, $[M - CHO{=}LeuO]^{+}$), 509 (1,5, $[M - MeC_6H_4SO_3H]^{+}$). Anal. calc. for $C_{37}H_{63}NO_8S$ (681.97): C 65.17, H 9.31, N 2.05, S 4.70; found: C 65.14, H 9.45, N 2.06, S 4.57.

Data of **7**: IR: 2145 (isocyano), 1743 (ester), 1598, 1496, 818 (aryl), 1371, 1176 (SO_2-aryl). CI-MS: (MeOH/H_2O 8:2 + 0.1M NH_4OAc, 1 ml/min): 681 (23, $[M + NH_4]^{+}$), 527 (45, $[M + NH_4^+ - MeC_6H_4SO_3^- + NH_3]^{+\cdot}$).

Data of **5a**: $[\alpha]_D^{20} = -8.7$, $[\alpha]_{546}^{20} = -10.0$ ($c = 1.0$, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 0.87, 0.88 (*t*-like, $J = 7$, 2 *Me*CH_2); 0.97, 0.98 (*d*, $J = 6$, *Me*$_2$CH); 1.08–1.40 (*m*, 12 CH_2, Me_2CH_2); 1.44–1.78 (*m*, 3 CH_2, Me_2CHCH_2); 1.90–2.10 (*m*, CH_2(4)); 2.45 (*s*, *Me*C_6H_4); 2.75 (*dt*, $J = 4$, 10, H–C(2)); 3.63 (*s*, MeO); 4.72 (*dt*, $J = 4$, 9, C(O)CHN); 4.77 (*dt*, $J = 4$, 6, irradiation at 2.75→*t*, $J = 6$, H–C(3)); 4.84 (*quint.*, $J = 6$, H–C(5)); 6.05 (*d*, $J = 9$, NH); 7.35, 7.80 (*d*, $J = 8$, 4 arom. H); 8.24 (*s*, CHO). Anal. calc. for $C_{37}H_{63}NO_8S$ (681.97): C 65.17, H 9.31, N 2.05, S 4.70; found: C 65.24, H 9.41, N 1.96, S 4.53.

Data of **6a**: ^{1}H-NMR ($CDCl_3$): 0.88, 0.89 (*t*-like, $J = 7$, 2 *Me*CH_2); 0.97, 0.98 (*d*, $J = 6$, *Me*$_2$CH); 1.05–1.47 (*m*, 12 CH_2, Me_2CH_2); 1.50–1.84 (*m*, 3 CH_2, Me_2CHCH_2); 1.90–2.14 (*m*, CH_2(4)); 2.45 (*s*, *Me*C_6H_4); 2.63–2.76 (*m*, H–C(2)); 3.68 (*s*, MeO); 4.55 (*quint.*, $J = 6$, H–C(5)); 4.73 (*dt*, $J = 5$, 9, C(O)CHN); 5.08–5.20 (*m*, H–C(3)); 6.06 (*d*, $J = 9$, NH); 7.35, 7.80 (*d*, $J = 8$, 4 arom. H); 8.24 (*s*, CHO). Anal. calc. for $C_{37}H_{63}NO_8S$ (681.97): C 65.17, H 9.31, N 2.05, S 4.70; found: C 65.32, H 9.45, N 2.13, S 4.56.

1.3. *Methyl (2S,3S,5S)-5-[(S)-2-Formamido-4-methylpentanoyloxy]-2-hexyl-3-(methylsulfonyloxy)hexadecanoate* (**5b**) *and Methyl (2S,3S,5S)-3-[(S)-2-Formamido-4-methylpentanoyloxy]-2-hexyl-5-(methylsulfonyloxy)hexadecanoate* (**6b**). A soln. of **2b**/**4** (4.13 g, 7.83 mmol), (i-Pr)$_2$EtN (1.77 g, 13.7 mmol), and 4-(dimethylamino)pyridine (120 mg) in pentane (20 ml) was cooled to 0° and MsCl (1.12 g, 9.78 mmol) added dropwise in 3 min. The mixture was diluted with Et_2O (10 ml) and stirred at r.t. for 5 h. Then further (i-Pr)$_2$EtN (0.45 g, 3.5 mmol) and MsCl (0.28 g, 2.45 mmol) were added, and the turbid mixture was stirred for additional 15 h. The suspension was diluted with Et_2O, H_2O added, the org. phase separated and washed twice with buffer soln. of pH 4 and brine, the neutral org. phase dried and evaporated, and the residue submitted to FC (silica gel, hexane/AcOEt 6:4): 3.46 g (73%) of **5b**/**6b** 2:1. Repetitive FC (hexane/AcOEt 6:4) provided pure **5b** and **6b** as colorless oils.

Data of **5b**: IR: 3365 (NH), 1739 (ester), 1677, 1517 (amide), 1356, 1175 (SO_2-aryl). ^{1}H-NMR ($CDCl_3$): 0.88 (*t*-like, $J = 7$, 2 *Me*CH_2); 0.97 (*d*, $J = 6$, *Me*$_2$CH); 1.14–1.40 (br. *s*, 12 CH_2, Me_2CH_2); 1.45–1.80 (*m*, 3 CH_2, Me_2CHCH_2); 2.06 (*t*-like, $J = 6$, CH_2(4)); 2.83 (*dt*, $J = 5$, 10, H–C(2)); 3.04 (*s*, $MeSO_2$); 3.72 (*s*, MeO); 4.71 (*dt*, $J = 5$, 9 C(O)CHN); 4.85 (*dt*, $J = 5$, 6, H–C(3)); 5.05 (*quint.*, $J = 6$, H–C(5)); 6.00 (*d*, $J = 9$, NH); 8.22 (*s*, CHO). MS: no $M^{+\cdot}$, 510 (1.5, $[M - MeSO_3]^{+}$). FAB-MS: 606 (3, $[M + H]^{+}$), 510 (10, $[M - MeSO_3]^{+}$). Anal. calc. for $C_{31}H_{59}NO_8S$ (605.87): C 61.46, H 9.82, N 2.31, S 5.29; found: C 61.18, H 9.70, N 2.30, S 5.23.

Data of **6b**: IR: 3371 (NH), 1743 (ester), 1687, 1519 (amide), 1353, 1174 (SO_2-aryl). ^{1}H-NMR ($CDCl_3$): 0.88 (*t*-like, $J = 7$, 2 *Me*CH_2); 0.96 (*d*, $J = 6$, *Me*$_2$CH); 1.14–1.37 (br. *s*, 12 CH_2, Me_2CH_2); 1.37–1.82 (*m*, 3 CH_2, Me_2CHCH_2); 1.96–2.21 (*m*, CH_2(4)); 2.76 (*dt*, $J = 5$, 10, H–C(2)); 3.05 (*s*, $MeSO_2$); 3.69 (*s*, MeO); 4.60–4.76 (*m*, C(O)CHN, H–C(3)); 5.15–5.26 (*m*, H–C(5)); 5.98 (*d*, $J = 9$, NH); 8.21 (*s*, CHO). MS: no $M^{+\cdot}$, 509 (1.5, $[M - MeSO_3H]^{+}$). Anal. calc. for $C_{31}H_{59}NO_8S$ (605.87): C 61.46, H 9.82, N 2.31, S 5.29; found: C 61.38, H 9.86, N 2.28, S 5.13.

1.4. *Methyl (2S,3R,5S)-5-[(S)-2-Formamido-4-methylpentanoyloxy]-2-hexyl-3-(nitryloxy)hexadecanoate* (**8**). A soln. of **5b** (0.56 g, 0.92 mmol) and $(Bu_4N)NO_3$ (0.84 g, 2.77 mmol) in toluene (8 ml) was refluxed for 13 h. Then the mixture was cooled to r.t., washed with H_2O, the aq. phase extracted 3× with Et_2O, and the pooled org. extract washed with brine, dried, and evaporated. FC (silica gel, hexane/AcOEt 75:25) yielded 142 mg (27%) of **8**. Yellowish oil. IR: 3307 (NH, OH), 1741 (ester), 1671, 1525 (amide), 1640, 1278, 856 (ONO_2). ^{1}H-NMR ($CDCl_3$): 0.88 (*t*-like, $J = 7$, 2 *Me*CH_2); 0.98 (*d*, $J = 6$, *Me*$_2$CH); 1.14–1.42 (br. *s*, 12 CH_2, Me_2CH_2); 1.42–1.82 (*m*, 3 CH_2, Me_2CHCH_2); 1.93 (*ddd*, $J = 4$, 10, 16, CH_2(4)); 2.80 (*dt*, $J = 4$, 6, H–C(2)); 3.72 (*s*, MeO); 4.70 (*dt*, $J = 5$, 9, C(O)CHN); 4.95–5.09 (*m*), 5.11–5.23 (*m*, 2 CH–O); 5.94 (*d*, $J = 9$, NH); 8.22 (*s*, CHO). MS: no $M^{+\cdot}$, 414 (0.7, $[M - CHO{-}LeuO]^{+}$), 351 (1.7, $[M - HNO_3 - CHO{-}LeuO]^{+\cdot}$). Anal. calc. for $C_{30}H_{56}N_2O_8$ (572.78): C 62.91, H 9.86, N 4.89; found: C 62.86, H 9.95, N 5.07.

1.5. *Methyl (2S,3S,5R)-3-[(S)-2-Formamido-4-methylpentanoyloxy]-2-hexyl-5-(nitryloxy)hexadecanoate* (**9**). With **6b** (272 mg, 0.45 mmol) following the procedure for **8**: 143 mg (56%) of **9**. Yellowish oil. IR: 3338 (NH,

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[4]) The amount of **7** formed varied strongly with the reaction conditions. With a catalytic amount of 4-(dimethylamino)pyridine and Et_3N as HCl quencher, 25–50% of the isolated product was **7**.

OH), 1744 (ester), 1689, 1521 (amide), 1631, 1277, 866 (ONO_2). ^{1}H-NMR ($CDCl_3$): 0.88 (*t*-like, $J = 7$, 2 $MeCH_2$); 0.96 (*d*, $J = 6$, Me_2CH); 1.20–1.40 (br. *s*, 12 CH_2, Me_2CH); 1.40–1.80 (*m*, 3 CH_2, Me_2CHCH_2); 1.83–2.06 (*m*, CH_2(4)); 2.65 (*dt*, $J = 5$, 10, H–C(2)); 3.70 (*s*, MeO); 4.70 (*dt*, $J = 5$, 9, C(O)CHN); 4.94–5.08 (*m*), 5.26–5.37 (*m*, 2 CH–O); 5.94 (*d*, $J = 9$, NH); 8.22 (*s*, CHO). MS (ion spray): 595 (76, $[M + Na]^+$), 590 (65, $[M + NH_4]^+$), 573 (100, $[M + H]^+$), 510 (54, $[M + H - HNO_3]^+$), 351 (90, $[M + H - HNO_3 - CHO–LeuOH]^+$). Anal. calc. for $C_{30}H_{56}N_2O_8$ (572.78): C 62.91, H 9.86, N 4.89; found: C 63.22, H 10.01, N 4.71.

1.6. *Methyl (2*S*,3*R*,5*S*)-5-[(*S*)-2-Formamido-4-methylpentanoyloxy]-2-hexyl-3-hydroxyhexadecanoate* (**3b**). Hydrogenolysis of a soln. of **8** (73 mg, 0.127 mmol) in i-PrOH (8 ml) in presence of 10% Pd/C (8 mg) at 10 bar H_2 for 2 h at r.t. provided, after usual workup and FC (silica gel, hexane/AcOEt 6:4), 46 mg (69%) of **3b**. Colorless oil. IR: 3348 (NH, OH), 1736 (ester), 1669, 1529 (amide). ^{1}H-NMR ($CDCl_3$): 0.88 (*t*-like, $J = 7$, 2 $MeCH_2$); 0.97 (*d*, $J = 6$, Me_2CH); 1.13–1.38 (br. *s*, 12 CH_2, Me_2CH); 1.46–1.80 (*m*, 3 CH_2, Me_2CHCH_2, CH_2(4)); 2.46 (*q*, $J = 7$, H–C(2)); 2.97 (br., OH); 3.58–3.75 (*m*, H–C(3)); 3.70 (*s*, MeO); 4.72 (*dt*, $J = 5$, 9, C(O)CHN); 5.06–5.20 (*m*, H–C(5)); 6.02 (*d*, $J = 9$, NH); 8.22 (*s*, CHO). MS: no M^+, 370 (3.6, $[M - C_6H_{13}CHCO_2Me]^+$). Anal. calc. for $C_{30}H_{57}NO_6$ (527.79): C 68.27, H 10.89, N 2.65; found: C 67.98, H 11.02, N 2.58.

1.7. *Methyl (2*S*,3*S*,5*R*)-3-[(*S*)-2-Formamido-4-methylpentanoyloxy]-2-hexyl-5-hydroxyhexadecanoate* (**10**). Hydrogenolysis of **9** (66 mg, 0.12 mmol) following the procedure for **3b** provided 46 mg (75%) of **10**. Colorless oil. IR: 3362 (NH, OH), 1742 (ester), 1671, 1527 (amide). ^{1}H-NMR ($CDCl_3$): 0.88 (*t*-like, $J = 7$, 2 $MeCH_2$); 0.96 (*d*, $J = 6$, Me_2CH); 1.13–1.37 (br. *s*, 12 CH_2, Me_2CH); 1.37–1.82 (*m*, 3 CH_2, $MeCHCH_2$, CH_2(4)); 2.6 (br., OH); 2.61–2.74 (*m*, H–C(2)); 3.38–3.54 (*m*, H–C(5)); 3.68 (*s*, MeO); 4.66 (*dt*, $J = 6$, 9, C(O)CHN); 5.27–5.40 (*m*, H–C(3)); 5.96 (*d*, $J = 9$, NH); 8.21 (*s*, CHO). MS: no M^+, 370 (4, $[M - C_6H_{13}CHCO_2Me]^+$), 352 (6, $[M - C_6H_{13}CHCO_2Me - H_2O]^+$). Anal. calc. for $C_{30}H_{57}NO_6$ (527.79): C 68.27, H 10.89, N 2.65; found: C 68.10, H 11.08, N 2.51.

1.8. *(3*S*,4*S*,6*S*)-3-Hexyltetrahydro-2-oxo-6-undecyl-2*H*-pyran-4-yl* N-*Formyl-*L*-leucinate* (**12**). *From (3*S*,4*S*,6*R*)-3-Hexyltetrahydro-4-hydroxy-6-undecyl-2*H*-pyran-2-one*: To a stirred soln. of *N*-formyl-L-leucine (0.318 g, 2 mmol) in THF (5 ml) was added 1,1′-carbonyldiimidazole (0.36 g, 2.2 mmol) in portions. After 1 h at r.t., (3*S*,4*S*,6*R*)-3-hexyltetrahydro-4-hydroxy-6-undecyl-2*H*-pyran-2-one [10] (0.708 g, 2 mmol) was added and the soln. stirred at r.t. for further 2 h. The solvent was evaporated at 30°, the residue dissolved in Et_2O, the org. phase washed 3× with H_2O, dried, and evaporated, and the resulting crystalline product further purified by FC (silica gel, hexane/AcOEt 2:1): 0.31 g (31%) of **12** and 0.23 g of starting pyranone with m.p. 105–107° ([10]: 95–97°). HPLC: (t_R 0.61). **12**: Colorless crystals. M.p. 108–110°. HPLC: t_R 0.76. $[\alpha]_D^{20} = +40.2$ ($c = 1.0$, $CHCl_3$). IR: 3348 (NH), 1734 (lactone), 1657, 1513 (amide). ^{1}H-NMR ($CDCl_3$): 0.88 (*t*-like, $J = 7$, 2 $MeCH_2$); 0.95 (*d*, $J = 5$, Me_2CH); 1.16-1.43 (br. *s*), 1.43–1.79 (*m*, 15 CH_2, Me_2CHCH_2); 1.79–1.95 (*m*, H_{ax}–C(5)); 2.44–2.65 (*m*, H–C(3), H_{eq}–C(5)); 4.13–4.30 (*m*, H–C(6)); 4.65 (*dt*, $J = 5$, 8, C(O)CHN); 5.40 (*dt*, $J = 4$, 10, H–C(4)); 5.92 (*d*, $J = 8$, NH); 8.20 (*s*, CHO). MS: 496 (9, $[M + H]^+$). Anal. calc. for $C_{29}H_{53}NO_5$ (495.75): C 70.26, H 10.78, N 2.83; found: C 70.07, H 10.62, N 2.81.

From **10**: A soln. of **10** (7 mg, 0.013 mmol) and TsOH (1 mg) in MeCN was stirred at r.t. for 2.5 d. Then the soln. was diluted with hexane, washed with sat. aq. $NaHCO_3$ soln. and brine, dried, and evaporated: 7 mg (quant.) of **12**. Colorless crystals. M.p. 103–107°. Mixed m.p. with sample of **12** obtained above, 106–110°. Mixed m.p. with recrystallized (3*S*,4*S*,6*R*)-3-hexyltetrahydro-4-hydroxy-6-undecyl-2*H*-pyran-2-one [10] (from Et_2O/hexane, m.p. 107–108°), 90–107°; HPLC: t_R 0.61.

1.9. *Methyl (2*S*,3*S*,5*S*)-2-Hexyl-3,5-dihydroxyhexadecanoate* (**17**). To a soln. of (2*S*,3*S*,5*S*)-2-hexyl-3,5-dihydroxyhexadecanoic acid (**14**; 50 mg, 0.134 mmol) [7] in Et_2O (4 ml) was added a soln. of CH_2N_2 in Et_2O until a persisting yellow color was obtained. After 30 min, the soln. was evaporated and the residue purified by FC (silica gel, hexane/AcOEt 4:1): 34 mg (66%) of **17** as a colorless oil which crystallized in the freezer. Recrystallization from pentane provided colorless crystals. M.p. 29.5–30.5°. $[\alpha]_D^{20} = -4.2$, $[\alpha]_{546}^{20} = -4.8$ ($c = 1.0$, $CHCl_3$). IR: 3476 (OH), 1728, 1709 (ester). ^{1}H-NMR ($CDCl_3$): 0.88 (*t*-like, $J = 7$, 2 $MeCH_2$); 1.15–1.37 (br. *s*), 1.37–1.79 (*m*, 15 CH_2, CH_2(4)); 2.46 (*dt*, $J = 5$, 9, H–C(2)); 3.38 (br., 2 OH); 3.73 (*s*, MeO); 3.79–3.93 (*m*, H–C(5)); 3.98 (*ddd*, $J = 2.5$, 3, 6, H–C(3)). MS: no M^+, 337 (0.6, $[M - H_2O - MeO]^+$). Anal. calc. for $C_{23}H_{46}O_4$ (386.62): C 71.45, H 11.99; found: C 71.52, H 11.78.

1.10. *Methyl (2*S*,3*R*,5*S*)-2-Hexyl-3,5-dihydroxyhexadecanoate* (**20**). To a soln. of potassium (2*S*,3*R*,5*S*)-2-hexyl-3,5-dihydroxyhexadecanoate (0.15 g, 0.37 mmol) [7] in MeOH (10 ml) was added MeI (1.14 g, 8 mmol) and the mixture stirred at r.t. for 3.5 d. Then further MeI (1.14 g, 8 mmol) was added and stirring at r.t. continued for additional 3 d. Then the soln. was evaporated, the residue dissolved in Et_2O, washed with H_2O and brine, dried, and evaporated. The crude product was purified by FC (silica gel, hexane/AcOEt 3:1): 77 mg (55%) of **20**. Colorless waxy product. M.p. 44.5–46°. Recrystallization from pentane provided colorless crystals. M.p. 54.5–55°. $[\alpha]_D^{20} = -0.7$, $[\alpha]_{546}^{20} = -1.0$ ($c = 0.6$, $CHCl_3$). IR (nujol): 3295 (OH); 1733 (ester). ^{1}H-NMR ($CDCl_3$): 0.88

(*t*-like, $J = 6$, 2 *Me*CH_2); 1.18–1.40 (br. *s*), 1.40–1.60 (*m*), 1.60–1.78 (*m*, 15 CH_2, CH_2(4)); 2.5 (br., 2 OH); 2.53 (*dt*, $J = 6$, 8, H–C(2)); 3.70 (*s*, MeO); 3.88–4.02 (*m*, H–C(5)); 4.10 (*ddd*, $J = 2$, 3, 6, H–C(3)). CI-MS (MeOH/H_2O 6:4 + 0.1M NH_4OAc, 1.0 ml/min): 404 (12, $[M + NH_4]^+$), 387 (100, $[M + H]^+$). Anal. calc. for $C_{23}H_{46}O_4$ (386.62): C 71.45, H 11.99; found: C 71.60, H 12.07.

2. *Incubations.* 2.1. *Incubation of [3′-^{14}C]-***1** *with HCEL and Separation of the Products by TLC* (see *Table 1*). To 200 ml of buffer pH 7.0 (5 mM in taurocholate and taurodeoxycholate, 1 mM in *Tris*-HCl, 1 mM in $CaCl_2$, 150 mM in NaCl with 0.02% NaN_3) was added [3′-^{14}C]-**1** (1 mg) in DMSO (2 ml) and stirred for 10 min at +37°. Then pure HCEL [2] (5 ml, 1.6 mg/ml) was added. An identical incubation without HCEL was used as blank. Samples of 200 µl were taken after 0, 1, 6, 24, and 48 h. The 200-µl samples were diluted with 1% KH_2PO_4 (1.05 ml) and MeOH/$CHCl_3$/heptane 1.41:1.25:1 (3.25 ml). After mixing and centrifugation 100 µl of each the upper and lower phase were taken for radioactivity determination. 1-ml samples of the lower org. phase, containing ≥ 99% of radioactivity (*cf. Table 2*), were evaporated, dissolved in 20 µl of $CHCl_3$/MeOH 2:1 and 10 µl thereof separated on TLC (silica gel, $CHCl_3$/acetone/AcOH 96:4:0.5). The plates were developed by phosphomolybdate and cut into five parts which were counted for radioactivity. After 48 h, the rest of the incubation soln. was lyophilized, extracted, and the phases were separated (see *Table 1*, after workup). The org. phases obtained were evaporated at r.t. and sent in dry ice to *F. Hoffmann-La Roche Ltd.* in Basel, Switzerland, for further processing.

2.2. *Incubation of [$^3H_4$]-***1**/*[1″-^{14}C]-***1** *with HCEL.* In an experiment of the same design as *2.1*, [$^3H_4$]-**1**/[1″-^{14}C]-**1** was incubated with HCEL and the ratio between ^3H and ^{14}C in the upper and lower phases determined. Results: *Table 2*.

3. *Methyl Esters of the Degradation Products.* The samples obtained from the University of Lund, Lund, Sweden, were dissolved in MeOH and treated with a soln. of CH_2N_2 in Et_2O until the yellow color persisted. After 18 h at r.t., the still yellow mixture was evaporated at r.t., and the resulting methylated derivatives were submitted to physico-chemical analysis.

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