Synthesis of Lipophilic Aldehydes and Study of their Inhibition Effect on Human Digestive Lipases

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Experimental procedures and characterization data

Methyl (4*S*)-2,2-dimethyl-1,3-dioxolane-4-carboxylate and AcNH-TEMPO were purchased from Aldrich. 1,2-Dicaprin was purchased from Sigma. Analytical TLC plates (silica gel 60 F_{254}) and silica gel 60 (70-230 mesh) were purchased from Merck. Visualization of spots was effected with UV light and/or phosphomolybdic acid and/or ninhydrin both in ethanol stain. HPL and HGL were purified at the laboratory using previously described procedures. Et_2O was dried by standard procedures and stored over Na. Et_3N was distilled over ninhydrin. All other solvents and chemicals were of reagent grade and used without further purification. Melting points were determined on a Buchi 530 apparatus and are uncorrected. ^1H NMR, and ^{13}C NMR spectra were obtained in $CDCl_3$ using a Varian Mercury spectrometer.

Etherification of 3-(benzyloxy)-1,2-propanediol.

Compound **1** (18 g, 100 mmol) was added to a stirred solution of 50% •aOH (500 mL), benzene (500 mL), Bu_4NHSO_4 (8.5 g, 25 mmol) and 1-bromododecane (130 mL, 0.6 mol). After vigorous stirring for 4 h at 45-50°C, the reaction mixture was allowed to obtain the ambient temperature and EtOAc and water were added. The organic phase was washed with brine and dried (Na_2SO_4). The products were separated by column chromatography (petroleum ether 40-60°C, petroleum ether 40-60°C/EtOAc 12/1, petroleum ether 40-60°C/EtOAc 7/3).

1-(benzyloxy)-3-dodecyloxypropan-2-ol (2).

Yield 14 g (40%); ^1H NMR: δ 7.35 (5H, m, C_6H_5), 4.60 (2H, s, $CH_2C_6H_5$), 4.00 (1H, m, $CHOH$), 3.75-3.38 (6H, m, 3×CH_2O), 1.62 (2H, m, CH_2CH_2O), 1.42-1.18 (18H, m, 9×CH_2), 0.88 (3H, t, J=6.2 Hz, CH_3); ^{13}C NMR: δ 137.9, 128.2, 127.5, 127.2, 73.2, 71.7, 71.5, 71.3, 69.4, 31.8, 29.5, 25.9, 22.5, 14.0; MS (FAB): m/z (%): 373 (100) [M+Na^+], 351 (32) [M+H^+]; Anal. Calcd. for $C_{22}H_{38}O_3$: C, 75.38, H, 10.93. Found C, 75.14, H, 10.99.

1-[(2,3-didodecyloxy)methyl]benzene (3).

Yield 13 g (26%); ^1H NMR: δ 7.35 (5H, m, C_6H_5), 4.57 (2H, s, $CH_2C_6H_5$), 3.62-3.41 (9H, m, CH, 4×CH_2O), 1.58 (4H, m, 2×CH_2CH_2O), 1.38-1.18 (36H, m, 18×CH_2), 0.9 (6H, t, J=6.8 Hz, 2×CH_3); ^{13}C NMR: δ 138.4, 128.2, 127.4, 127.1, 77.8, 73.3, 71.6, 70.7, 70.5, 70.2, 31.9, 30.0, 29.6, 29.5, 29.3, 26.1, 22.7, 14.0; MS (FAB): m/z (%): 519 (22) [M+H^+], 91 (100); Anal. Calcd. for $C_{34}H_{62}O_3$: C, 78.80, H, 12.04. Found C, 78.52, H, 12.31.

2-(benzyloxy)-1-(dodecyloxymethyl)ethyl decanoate (4).

To a stirred solution of compound **2** (14 g, 40 mmol), decanoic acid (6.9 g, 40 mmol) and DMAP (49 mg, 0.4 mmol) in CH_2Cl_2 (200 mL), DCC (9.9 g, 48 mmol) in CH_2Cl_2 (80 mL) was added dropwise at 0°C. After being kept at 0°C for 30 min, the reaction mixture was stirred at room temperature for 24 h, then filtered to remove the white precipitate, concentrated under reduced pressure and purified by column chromatography (petroleum ether 40-60°C/EtOAc 9/1).

Yield 20 g (89%); ^{1}H NMR: δ 7.35 (5H, m, C_6H_5), 5.20 (1H, m, CHOCO), 4.58 (2H, s, $CH_2C_6H_5$), 3.64 (4H, m, 2×CH_2O), 3.41 (2H, m, CH_2O), 2.35 (2H, t, J=7.4 Hz, CH_2CO), 1.60 (4H, m, CH_2CH_2CO, CH_2CH_2O), 1.44-1.18 (30H, m, 15×CH_2), 0.9 (6H, t, J=6.0 Hz, 2×CH_3); ^{13}C NMR: δ 173.3, 138.0, 128.2, 127.7, 127.2, 73.1, 71.5, 71.1, 69.1, 68.7, 34.4, 31.8, 29.6, 29.4, 29.2, 29.0, 26.0, 22.6, 14.0; Anal. Calcd. for $C_{32}H_{56}O_4$: C, 76.14, H, 11.18. Found C, 76.48, H, 11.25.

General procedure for the removal of the benzyl group.

To a solution of compound **3** or **4** (1 mmol) in EtOH (2.5 mL), through which N_2 had been passed or 5 min, 10% Pd/C catalyst (0.042 g) was added. The reaction mixture stirred under H_2 for 5 h at room temperature. The catalyst was removed by filtration through a pad of Celite and the filtrate was evaporated under reduced pressure. The product was purified by column chromatography (petroleum ether 40-60°C/EtOAc 8/2).

2,3-bis(dodecyloxy)propan-1-ol (5).

Yield 0.32 g (75%); mp 37-39°C; ^{1}H NMR: δ 3.68-3.37 (9H, m, 3×CH_2O, CH_2OH, CH), 1.55 (4H, m, 2×CH_2CH_2O), 1.38-1.15 (36H, m, 18×CH_2), 0.88 (6H, t, J=6.0 Hz, 2×CH_3); ^{13}C NMR: δ 77.6, 72.0, 71.0, 70.6, 63.2, 31.9, 29.8, 29.6, 29.5, 29.3, 26.1, 22.6, 14.0; Anal. Calcd. for $C_{27}H_{56}O_3$: C, 75.64, H, 13.17. Found C, 75.99, H, 13.51.

2-hydroxy -1-(dodecyloxymethyl)ethyl decanoate (6).

Yield 0.24 g (58%); mp 34-36°C; ^{1}H NMR: δ 4.98 (1H, m, CHOCO), 3.79 (2H, d, J=6.0 Hz, CH_2OH), 3.60 (2H, d, J=6.0 Hz, $CHCH_2O$), 3.4 (2H, t, J=6.0 Hz, CH_2O), 2.35 (2H, t, J=7.4 Hz, CH_2CO), 1.58 (4H, m, CH_2CH_2CO, CH_2CH_2O), 1.45-1.15 (30H, m, 15×CH_2), 0.88 (6H, t, J=6.0 Hz, 2× CH_3); ^{13}C NMR: δ 173.7, 72.9, 71.8, 69.9, 62.8, 34.3, 31.8, 29.6, 29.4, 29.3, 29.2, 29.0, 26.0, 25.0, 22.6, 14.1; Anal. Calcd. for $C_{25}H_{50}O_4$: C, 72.41, H, 12.15. Found C, 72.79, H, 12.52.

General procedure for the preparation of the unsaturated compounds 7, 8.

To a solution of compound **5** or **6** (1.0 mmol), in a mixture of EtOAc/toluene 1:1 (6 mL), a solution of NaBr (0.12 g, 1.1 mmol) in water (0.5 mL) and subsequently AcNH-TEMPO (2 mg, 0.01 mmol)

were added at -10 °C. To the resulting biphasic system was added under vigorous stirring a solution of NaOCl (0.08 g, 1.1 mmol) and $NaHCO_3$ (0.08 g, 1.0 mmol) in H_2O (0.7 mL) dropwise at -10 °C over a period of 15 min. After stirring at –10 °C for 10 min, EtOAc (15 mL) and water (5 mL) were added. The organic layer was washed with 1% aqueous citric acid (10 mL), which contained KI (0.5 g), 10% aqueous $Na_2S_2O_3$ (10 mL), brine and dried over Na_2SO_4. The solvent was evaporated under reduced pressure and the aldehyde was used directly to the next step without any purification.
To a solution of the aldehyde (0.21 g, 1.0 mmol) in dry THF (5 mL), Ph_3P=CHCOOBu^t (0.37 g, 1.1 mmol) was added and the reaction mixture was refluxed for 1 h. The solvent was evaporated under reduced pressure and the residue was purified by column chromatography (petroleum ether 40-60°C/EtOAc 85/15).

***tert*-butyl (*E*)-4,5-bis(dodecyloxy)-2-pentenoate (7).**

Yield 0.42 g (80%); ^{1}H NMR: δ 6.75 (1H, dd, *J*=15.8 Hz, *J*=5.8 Hz, C*H*=CHCOO), 5.97 (1H, dd, *J*=15.8 Hz, *J*=1.1 Hz, CH=C*H*COO), 4.02 (1H, m, CH), 3.56-3.35 (6H, m, 3×CH_2O), 1.60-1.44 (13H, m, 2×CH_2CH_2O, $C(CH_3)_3$), 1.36-1.13 (36H, m, 18×CH_2), 0.88 (t, 6H, *J*=6.6 Hz, 2×CH_3); ^{13}C NMR: δ 165.5, 142.6, 124.2, 80.4, 78.2, 72.9, 71.7, 70.1, 31.9, 29.8, 29.6, 29.4, 29.3, 28.1, 28.0, 26.0, 22.6, 14.0; MS (FAB): m/z (%): 547(5) [M+Na$^+$], 57 (100); Anal. Calcd. for $C_{33}H_{64}O_4$: C, 75.52, H, 12.29. Found C, 75.78, H, 12.01.

***tert*-butyl (*E*)-4-(decanoyloxy)-5-(dodecyloxy)-2-pentenoate (8).**

Yield 0.29 g (56%); ^{1}H NMR: δ 6.76 (1H, dd, *J*=15.4 Hz, *J*=5.9 Hz, C*H*=CHCOO), 5.91 (1H, dd, *J*=15.4 Hz, *J*=1.2 Hz, CH=C*H*COO), 5.58 (1H, m, CHOCO), 3.59-3.38 (4H, m, 2×CH_2O), 2.37 (2H, t, *J*=7.4 Hz, CH_2CO), 1.76-1.38 (13H, m, $C(CH_3)_3$, 2×CH_2), 1.37-1.17 (30H, m, 15×CH_2), 0.88 (6H, t, *J*=6.0 Hz, 2×CH_3); ^{13}C NMR: δ 172.8, 165.0, 141.6, 124.4 80.6, 71.6, 71.3, 70.8, 34.3, 31.8, 29.6, 29.4, 29.2, 28.1, 28.0, 26.0, 24.8, 22.6, 14.1; MS (FAB): m/z (%): 533 (15) [M+Na$^+$], 57 (100); Anal. Calcd. for $C_{31}H_{58}O_5$: C, 72.89, H, 11.45. Found C, 72.63, H, 11.74.

General procedure for the preparation of the carboxylic acids 9, 10.

To a solution of compound **7** or **8** (1 mmol) in EtOH (2.5 mL), through which N_2 had been passed for 5 min, 10% Pd/C catalyst (0.04 g) was added. The reaction mixture stirred under H_2 for 24 h at room temperature. The catalyst was removed by filtration through a pad of Celite and the filtrate was evaporated under reduced pressure. The product was purified by column chromatography (petroleum ether 40-60°C/EtOAc 9/1) and then treated with TFA (3.5 mL) in CH_2Cl_2 (3.5 mL) for 1 h at room temperature. The solvent and the excess acid were evaporated under reduced pressure and the residue was crystallized from Et_2O.

4,5-bis(dodecyloxy)pentanoic acid (9).

Yield 0.42 g (90%); mp 39-40°C; ^{1}H NMR: δ 3.62 (1H, m, CH), 3.43 (6H, m, 3×CH_2O), 2.48 (2H, t, J=7.2 Hz, CH_2COOH), 1.85 (2H, m, CH_2CH_2COOH), 1.55 (4H, m, 2×CH_2CH_2O), 1.40-1.09 (36H, m, 18×CH_2), 0.88 (6H, t, J=6.0 Hz, 2×CH_3); ^{13}C NMR: δ 179.5, 77.4, 73.0, 71.6, 70.4, 31.9, 30.0, 29.6, 29.5, 29.3, 28.1, 28.0, 26.1, 22.7, 14.0; MS (FAB): m/z (%): 493 (30) [M+Na^+], 471 (25) [M+H^+]; Anal. Calcd. for $C_{29}H_{58}O_4$: C, 73.99, H, 12.42. Found C, 74.11, H, 12.69.

4-(decanoyloxy)-5-(dodecyloxy)pentanoic acid (10).

Yield 0.41 g (90%); mp 42-43°C; ^{1}H NMR: δ 5.03 (1H, m, CHOCO), 3.41 (4H, m, 2×CH_2O), 2.37 (4H, m, 2×CH_2CO), 1.98 (2H, m, CH_2CH_2COOH), 1.54 (4H, m, CH_2CH_2COO, CH_2CH_2O), 1.41-1.02 (30H, m, 15×CH_2), 0.88 (6H, t, J=6.0 Hz, 2×CH_3); ^{13}C NMR: δ 176.4, 173.2, 71.3, 71.0, 65.6, 34.2, 31.7, 29.4, 29.3, 29.2, 29.1, 29.0, 25.6, 24.8, 22.5, 14.9; MS (FAB): m/z (%): 479 (30) [M+Na^+], 457 (35) [M+H^+]; Anal. Calcd. for $C_{27}H_{52}O_5$.0.5 H_2O: C, 69.63, H, 11.47. Found C, 69.55, H, 11.68.

General procedure for the preparation of compounds 11, 12.

To a stirred solution of compound **9** or **10** (1.0 mmol) in dry THF (5 mL) at –10°C, NMM (110 µL, 1.0 mmol) was added, followed by ClCOOEt (96 µL, 1.0 mmol). After 10 min, $NaBH_4$ (0.11 g, 3.0 mmol) was added in one portion. MeOH (10 mL) was then added dropwise to the mixture over a period of 10 min at 0°C. The solution was stirred for additional 10 min and then neutralized with 1M $KHSO_4$. The organic solvents were evaporated under reduced pressure and the product was extracted with EtOAc (3 x 7 mL). The organic phase was washed by 1M $KHSO_4$ (5 mL), H_2O (10 mL), 5% $NaHCO_3$ (5 mL), H_2O (10 mL), dried, and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography (petroleum ether 40-60°C/EtOAc 7/3).

4,5-bis(dodecyloxy)-1-pentanol (11).

Yield 0.34 g (75%); ^{1}H NMR: δ 3.67 (3H, m, CHO, CH_2OH), 3.52-3.34 (6H, m, 3×CH_2O), 1.72-1.44 (8H, m, 2×CH_2CH_2O, $CHCH_2CH_2CH_2OH$), 1.42-1.15 (36H, m, 18×CH_2), 0.90 (6H, t, J=6.0 Hz, 2×CH_3); ^{13}C NMR: δ 78.4, 73.0, 71.6, 70.2, 62.9, 31.9, 29.6, 29.5, 29.3, 26.1, 22.7, 14.0 .

1-(dodecyloxymethyl)-4-hydroxybutyl decanoate (12).

Yield 0.26 g (60%); ^{1}H NMR: δ 5.05 (1H, m, CHOCO), 3.67 (2H, t, J=6.8 Hz, CH_2OH), 3.52-3.39 (4H, m, 2×CH_2O), 2.32 (2H, t, J=7.1 Hz, CH_2CO), 1.81-1.48 (8H, m, $CHCH_2CH_2CH_2OH$,

CH_2CH_2O, CH_2CH_2COO,), 1.42-1.15 (30H, m, 15×CH_2), 0.88 (6H, t, J=6.0 Hz, 2×CH_3); ^{13}C NMR: δ 173.2, 73.4, 71.7, 71.5, 62.4, 34.2, 31.9, 29.6, 29.4, 29.2, 28.3, 26.0, 22.5, 14.1.

General procedure for the preparation of the aldehyde compounds 13, 14.

To a solution of compound **11** or **12** (1.0 mmol), in a mixture of EtOAc/toluene 1:1 (6 mL), a solution of NaBr (0.12 g, 1.1 mmol) in water (0.5 mL) and subsequently AcNH-TEMPO (2 mg, 0.01 mmol) were added at -10°C. To the resulting biphasic system was added under vigorous stirring a solution of NaOCl (0.08 g, 1.1 mmol) and $NaHCO_3$ (0.08 g, 1.0 mmol) in H_2O (0.7 mL) dropwise at -10°C over a period of 15 min. After stirring at –10°C for 10 min, EtOAc (15 mL) and water (5 mL) were added. The organic layer was washed with 1% aqueous citric acid (10 mL), which contained KI (0.5 g), 10% aqueous $Na_2S_2O_3$ (10 mL), brine and dried over Na_2SO_4. The solvent was evaporated under reduced pressure and the product was purified by column chromatography (petroleum ether 40-60°C/EtOAc 7/3).

4,5-bis(dodecyloxy)pentanal (13).

Yield 0.27 g (60%); ^{1}H NMR: δ 3.62-3.31 (7H, m, CH, 3×CH_2O), 2.54 (2H, t, J=7.3 Hz, CH_2CH=O), 1.92 (2H, m, CH_2CH_2CH=O), 1.55 (4H, m, 2×CH_2CH_2O), 1.42-1.19 (36H, m, 18×CH_2), 0.88 (6H, t, J=6.0 Hz, 2×CH_3); ^{13}C NMR: δ 202.4, 77.6, 72.7, 71.6, 70.3, 40.1, 31.9, 30.0, 29.6, 29.5, 29.3, 26.1, 25.0, 22.7, 14.1; MS (FAB): m/z (%): 455 (22) [M+H$^+$], 269 (48); Anal. Calcd. for $C_{29}H_{58}O_3$: C, 76.59, H, 12.85. Found C, 76.28, H, 13.11.

1-(dodecyloxymethyl)-4-oxobutyl decanoate (14).

Yield 0.32 g (72%); ^{1}H NMR: δ 4.97 (1H, m, CHOCO), 3.39 (4H, m, 2×CH_2O), 2.44 (2H, t, J=6.8 Hz, CH_2CH=O), 2.24 (2H, t, J=7.0 Hz, CH_2COO), 1.91 (2H, m, CH_2CH_2CH=O), 1.57 (4H, m, CH_2CH_2COO, CH_2CH_2O), 1.34-1.08 (30H, m, 15×CH_2), 0.88 (6H, t, J=6.0 Hz, 2×CH_3); ^{13}C NMR: δ 201.2, 173.4, 73.1, 71.6, 40.1, 34.4, 31.8, 29.6, 29.4, 29.3, 29.2, 29.1, 26.0, 25.0, 22.7, 14.1; MS (FAB): m/z (%): 463 (63) [M+Na$^+$], 441 (7) [M+H$^+$], 269 (100); Anal. Calcd. for $C_{27}H_{52}O_4$: C, 73.59, H, 11.89. Found C, 73.21, H, 11.99.

(1*R*)-1-[(dodecyloxy)methyl]-4-oxobutyl decanoate (21).

It was prepared starting from the optically active methyl (4*S*)-2,2-dimethyl-1,3-dioxolane-4-carboxylate through a similar course of reactions. Spectroscopic data identical to those obtained for compound **14**. $[\alpha]_D$ = 8.8 (c 0.5 $CHCl_3$).

Monomolecular Film Experiments

Reasons for Using Lipid Monolayers. There are at least five major reasons for using lipid monolayers as substrates for lipolytic enzymes: (i) It is easy to follow the course of the reaction monitoring one of several physicochemical parameters characteristic of the monolayer film: surface pressure, potential, density, etc. (ii) Probably the most important reason is that it is possible with lipid monolayers to vary and control the "interfacial quality", which depends on the nature of the lipids forming the monolayer, the orientation and conformation of the molecules, the molecular and charge densities, the water structure, the viscosity, etc. (iii) Using the surface barostat balance, the lipid packing of a monomolecular film of substrate can be maintained constant during the course of hydrolysis, and it is therefore possible to obtain accurate presteady state kinetic measurements with minimal perturbation caused by increasing amounts of reaction products. (iv) The monolayer technique is highly sensitive and very little lipid is needed to obtain kinetic measurements. This advantage can often be decisive in the case of synthetic or rare lipids. (v) Inhibition of lipase activity by water-insoluble substrate can be precisely estimated using the "zero-order" trough and mixed monomolecular films in the absence of any synthetic, non-physiological detergent. The monolayer technique is therefore suitable for modeling *in vivo* situations.

Force/area Curves. Surface pressure-molecular area curves were measured in the rectangular reservoir compartment of the "zero order" trough (14.8 cm wide and 24.9 cm long). Before each experiment the trough was at first washed with tap water, then gently brushed in the presence of distilled ethanol, washed again with plenty of tap water and finally rinsed with double-distilled water. The lipidic film as a solution in $CHCl_3$ (approximately 1 mg mL^{-1}), was spread with a Hamilton syringe over an aqueous subphase of Tris/HCl 10 mM, pH 8.0, NaCl 100 mM, $CaCl_2$ 21 mM, EDTA 1 mM. The above buffer solution was prepared with double-distilled water and filtered through a 0.22 μm Millipore membrane. Before each utilization, residual surface-active impurities were removed by sweeping and suction of the surface. The force/area curves were automatically recorded upon a continuous compression rate at 4.8 cm min^{-1}.

Enzymes Kinetics Experiments. The inhibition experiments were performed using the monolayer technique. The surface pressure of the lipid film was measured using the platinum Wilhelmy plate technique coupled with an electromicrobalance.

For the inhibition studies the method of "mixed monomolecular films" was used. This method involves the use of a "zero-order" trough, consisting of two compartments: a reaction compartment, where mixed films of substrate and inhibitor are spread, and a reservoir compartment, where only pure films of substrate are spread. The two compartments are connected to each other by narrow

surface channels. HPL (final concentration 7.5 ng mL^{-1}) and HGL (final concentration 84 ng mL^{-1}) were injected into the subphase of the reaction compartment, where efficient stirring was applied. In the case of HPL the aqueous subphase was composed of Tris/HCl 10 mM, pH 8.0, NaCl 100 mM, $CaCl_2$ 21 mM, EDTA 1 mM. In the case of HGL the aqueous subphase was composed of CH_3COONa/HCl 10 mM, pH 5.0, NaCl 100 mM, $CaCl_2$ 21 mM, EDTA 1 mM. When, due to the lipolytic action of the enzyme, the surface pressure decreased a mobile barrier was moving over the reservoir compartment to compress the film and thus keep the surface pressure constant. The surface pressure was measured on the reservoir compartment. The surface of the reaction compartment was 100 cm^2 and its volume 120 mL. The reservoir compartment was 14.8 cm wide and 24.9 cm long. The lipidic films were spread from a chloroform solution (approximately 1 mg mL^{-1}). The kinetics were recorded for 20 min. In all cases linear kinetics were obtained. Each experiment was duplicated.