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18-Iodooctadeca-(8*Z*,11*Z*)-dienoic Acid as Useful Intermediate for the Synthesis of Special Lipoxygenase Substrates Bearing Bulky Substituents at the ω-Position

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Abstract—18-Iodooctadeca-(8*Z*,11*Z*)-dienoic acid (**7**) was synthesized in five steps starting from methyl 10-bromodec-7-ynoate (**2**) in an overall yield of 53%. The synthetic procedure involves Cu(I)-catalyzed cross-coupling of propargylic bromide **2** with 7-octyn-1-ol (**3**), followed by hydrogenation of the coupling product **4** to *Z*,*Z*-diene **5** on Lindlar's catalyst and subsequent substitution of the OH– group of **5** with iodine. Coupling of the resulting iodide **7** with low-order organic cuprates [*t*-Bu_2CuLi or $(PhCH_2)_2CuMgCl$] leads to 19,19-dimethyleicosa-(8*Z*,11*Z*)-dienoic acid (**1a**) and 19-phenylnonadeca-(8*Z*,11*Z*)-dienoic acid (**1b**), respectively.

Lipoxygenases (LOXs) constitute a family of lipid peroxidizing enzymes, which are involved in the biosynthesis of inflammatory mediators, in cell differentiation and atherogenesis.[1] These enzymes have been well characterized with respect to their enzymatic[1] and molecular biological properties.[2] The crystal structures of three LOX isoforms have recently been solved[3] and the X-ray coordinates were used to construct models for enzyme/substrate interaction.[4] More recently colonies of knockout mice have been bred in which the genes coding several LOX isoforms were functionally disrupted.[5] Although there is a substantial body of experimental data as to the biological relevance of LOX,[6] the chemistry of the lipoxygenase reaction is less well investigated. Previous site directed mutagenesis studies[7] and experiments with synthetic arachidonic acid isomers[8] indicated that the methyl terminus of fatty acid substrates appears to be important for substrate binding. Introduction of a bulky group at the methyl end of the fatty acid molecules may alter the substrate alignment at the active site and thus, the reaction characteristics. In addition, a shift in the position of the double bonds in either direction altered the specificity of the oxygenase reaction and caused a modification of the product pattern.[8] We have recently synthesized a set of ω-hydroxylated fatty acid isomers and found them to be much less effective LOX substrates than naturally occurring polyunsaturated fatty acids (PUFA's).[9]

To find out whether steric constrains or alterations in the hydrophobicity of the methyl terminus may be responsible for this effect we synthesized two fatty acid derivatives [19,19-dimethyleicosa-(8*Z*,11*Z*)-dienoic acid (**1a**) and 19-phenylnonadeca-(8*Z*,11*Z*)-dienoic acid (**1b**)] bearing bulky residues at the methyl terminus of the fatty acid chain. For this purpose we developed a flexible synthetic procedure which allows the preparation of ω-modified fatty acids in the mg-scale. These compounds may further be used to investigate the detailed mechanism of the LOX reaction, in particular to answer the question of whether or not there may be an inverse head to tail substrate orientation.

The synthesis of **1a** and **1b** was accomplished *via* the formation of 18-iodooctadeca-(8*Z*,11*Z*)-dienoic acid (**7**) as crucial intermediate (Scheme 1). The diacetylenic ester **4** was prepared (87%) by Cu(I)-catalyzed cross-coupling of propargylic bromide **2**[10] and 7-octyn-1-ol (**3**) followed by purification of the reaction product using silica gel flash chromatography (Et_2O/hexane 2:1, argon atmosphere). The alcohol **3** was prepared by reduction of methyl 7-octinoate[11] with $LiBH_4$ in THF by a standard procedure. Stereospecific hydrogenation of the skipped-triple bonds of **4** with Lindlar's catalyst and quinoline in benzene resulted in crude **5** with 92% purity, as indicated by RP-HPLC. The reaction product was purified by preparative RP-HPLC resulting in pure **5** with an 84% yield. The hydroxy ester **5** was converted to the iodide **7** *via* intermediate formation of the corresponding chloride **6**. The complete substitution of chlorine to iodine which was controlled by RP-HPLC

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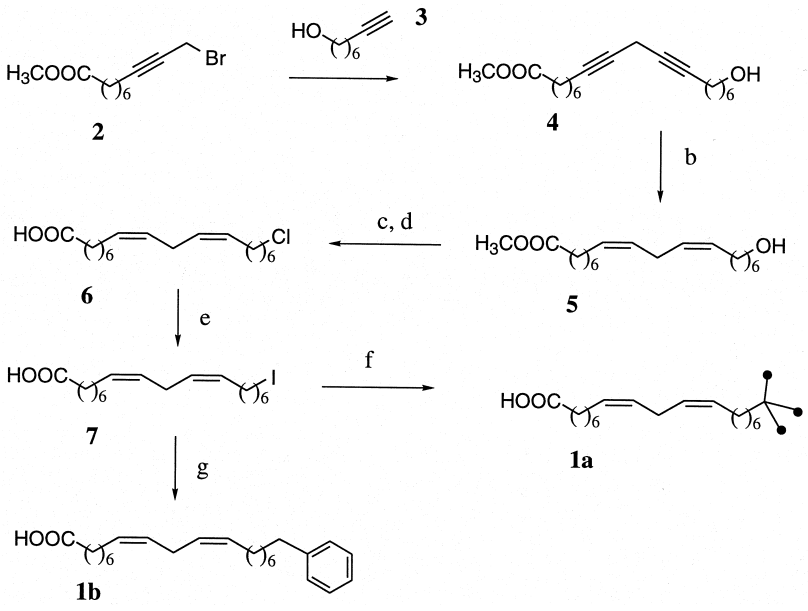

Scheme 1. (a) **3**, CuI, NaI, K_2CO_3, DMF, rt, 8 h; (b) H_2/Lindlar's catalyst, quinoline, benzene, 10°C; (c) Ph_3P, CCl_4, CH_2Cl_2, rt, 24 h; (d) LiOH, MeOH–H_2O, rt, 8 h; (e) NaI, acetone, 65°C, 20 h; (f) CuI, *t*-BuLi, THF, HMPA, −78 to −50°C, (g) CuI, $PhCh_2MgCl$, THF, HMPA, −50°C.

took about 20 h. Finally, either the tertiary alkyl (*t*-Bu_2CuLi) or benzyl [$(PhCH_2)_2CuMgCl$] organocuprates were coupled to the iodide **7** affording the acids **1a** and **1b** with a 79–82% yield. The low-order organocuprates were prepared in THF/HMPA from *t*-BuLi and $PhCH_2MgCl$, respectively. Formation of the organocuprate from the corresponding Grignard reagent required 1.5 h (−50°C) and preparation of *t*-Bu_2CuLi at −78°C took only about 35–45 min. Neither PhCu nor Ph_2CuLi have been alkylated by the iodide **7** even at elevated temperatures and no reaction has been observed with corresponding tosylate.

In conclusion, the synthesis of the key intermediate **7** provides an efficient and economic route for the preparation of ω-modified polyenoic fatty acids which can be used to further investigate the detailed mechanism of the LOX reaction, in particular the structural basis of enzyme/substrate interaction.

Experimental

General

^1H NMR and ^{13}C NMR spectra were recorded either on a Bruker MSL 200 or on a Bruker MSL 300 spectrophotometer in $CDCl_3$ as solvent. Chemical shifts are referenced to tetramethylsilane as an internal standard for ^1H NMR or to the deuterium lock signal of $CDCl_3$ ($\delta^{13}C$=77.24 ppm). IR spectra were recorded on Shimadzu IR-435. RP-HPLC analysis was carried out on a Shimadzu LC-10Avp liquid chromatograph connected to SPD-10Advp UV detector. Fatty acid analysis was performed on a Nucleosil C18-column; 150×4 mm, 5 μm particle size (Macherey–Nagel, Düren, Germany). A solvent system of MeOH/H_2O/AcOH (85:15:0.1, by vol.) and a flow rate of 1 mL/min were used for all compounds except for the final synthesis of product **1**. For this product a solvent system of MeOH/CH_3CN/H_2O (47.5:47.5:5, by vol.) and a flow rate of 1.2 mL/min were employed. Preparative purification was carried out on a Lichrospher 100 RP18 column; 250×22.5 mm, 10 μm particle size (Knauer, Berlin, Germany). For EIMS analysis a Shimadzu GC-MS QP-2000 system was used with an ion source temperature of 180°C and an electron energy of 70 eV. Column chromatography was carried out on silica gel 60 from Merck (Darmstadt, Germany), particle size ranging from 70–230 mesh. For thin-layer chromatography we employed precast silica gel 60 F254 sheets (Merck, Darmstadt, Germany). THF was freshly distilled from sodium/benzophenone ketyl, and HMPA was dried over CaH_2. All solvents and reagents used were of extra pure grade and purchased from Merck or Aldrich (Germany). *t*-Butyllithium (Merck) and benzylmagnesium chloride (Aldrich) were preliminarily titrated as described by Watson.[12] Prior to use, all glassware and syringes were dried at 140°C overnight and all reactions were carried out under atmosphere of dry argon.

Methyl 18-hydroxyoctadeca-8,11-diynoate (4). To a suspension of previously dried salts CuI (1.460 g, 7.66 mmol), NaI (1.149 g, 7.66 mmol) and K_2CO_3 (0.793 g, 5.74 mmol) in DMF (10 mL) the bromide **2** (1.100 g, 4.21 mmol) and alcohol **3** (0.483 g, 3.83 mmol) were added under argon atmosphere. After stirring for 8 h at rt, the mixture was quenched with sat. aq NH_4Cl (100 mL) and extracted with Et_2O (4×40 mL). The combined organic extracts were washed with sat. aq. NaCl (2×70 mL), dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (Et_2O/hexane 2:1) to afford **4** (1.021 g, 87%) as a colorless oil. IR (neat)/cm^{-1}: 3600–3200 (OH), 2170 (C≡C), 1740 (C=O). ^1H NMR (200 MHz, $CDCl_3$) δ: 3.65 (s, 3H, OCH_3), 3.62 (t, 2H, J=6.4 Hz, 18-CH_2), 3.22 (m, 2H, 10-CH_2), 2.36 (t, 2H, J=7.0 Hz, 2-CH_2), 2.14 (m, 4H, 7-CH_2 and 12-CH_2), 1.3–1.6

(m, 16H). Anal. calcd for $C_{19}H_{30}O_3$: C, 74.46; H, 9.87. Found C, 74.21; H, 9.59.

Methyl 18-hydroxyoctadeca-(5*Z*,8*Z*)-dienoate (5). Dry benzene (30 mL) was added to a 250-mL Erlenmeyer flask with Lindlar's catalyst (1.12 g). The mixture was saturated with H_2 at rt and cooled to 10°C. Then a solution of **4** (0.81 g, 2.64 mmol) in benzene (30 mL) and quinoline (1.1 mL) were added under a stream of Ar. After the Ar was exchanged with H_2, the reaction mixture was stirred for 1 h at 10°C, then filtered, washed with 2 M HCl (2×30 mL) and the solvent was evaporated. The crude residue was purified by preparative RP-HPLC (solvent system MeOH/H_2O, 9:1) to yield 0.69 g (84%) pure **5**. RP-HPLC RT=4.57 min. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 5.28–5.38 (m, 4H, CH=CH), 3.65 (s, 3H, OCH_3), 3.62 (t, 2H, J=6.4 Hz, 18-CH_2), 2.75 (m, 2H, 10-CH_2), 2.35 (t, 2H, J=7.0 Hz, 2-CH_2), 2.08 (m, 4H, 7-CH_2 and 13-CH_2), 1.63 (m, 4H, 3-CH_2 and 17-CH_2), 1.25–1.45 (m, 12H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 174.75, 130.16, 129.83, 128.27, 127.89, 62.79, 51.54, 34.09, 32.77, 29.64, 29.46, 29.09 (3C), 27.18 (2C), 25.67 (2C), 24.94. EIMS: *m*/*z* (%) 310 (0.05) [M^+], 278 (2.16) [M^+-CH_3OH]. Anal. calcd for $C_{19}H_{34}O_3$: C, 73.50; H, 11.04. Found C, 73.79; H, 11.30.

18-Chlorooctadeca-(8*Z*,11*Z*)-dienoic acid (6). To a solution of alcohol **5** (600 mg, 1.93 mmol) in CCl_4 (5 mL), a solution of PPh_3 (709 mg, 2.71 mmol) in CH_2Cl_2 (6 mL) was added at rt. After the reaction mixture was stirred for 24 h, the solvent was removed under reduced pressure and the residue was filtrated over silica gel (hexane/Et_2O, 1:1) affording 614 mg of a colorless oil as a product. The latter was reconstituted in methanol (60 mL) and added to an aqueous solution of LiO H (1.8 M, 15 mL) under Ar. The mixture was stirred at rt for 8 h. After the reaction was completed, methanol was removed by evaporation, the pH was adjusted carefully to 5.0 using diluted HCl (1 M) and the lipophilic compounds were extracted with Et_2O (3×40 mL). The combined organic extracts were dried over Na_2SO_4, concentrated under reduced pressure and the product was purified by silica gel chromatography (hexane/Et_2O, 1:2) yielding pure **6** (492 mg, 81%). TLC: R_f=0.35 (hexane/Et_2O, 1:1, with 1% AcOH). RP-HPLC: RT=3.42 min. IR (neat) ν: 3030, 1670 (CH=CH), 1705 (C=O), 720, 640 (C–Cl) cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 5.28–5.38 (m, 4H, CH=CH), 3.51 (t, 2H, J=6.8 Hz, 18-CH_2), 2.75 (m, 2H, 10-CH_2), 2.34 (t, 2H, J=7.1 Hz, 2-CH_2), 2.05 (m, 4H, 7-CH_2 and 13-CH_2), 1.61–1.80 (m, 6H, 3-CH_2, 16-CH_2 and 17-CH_2), 1.25–1.45 (m, 10H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 180.70, 130.24, 129.94, 128.44, 128.07, 45.33, 34.27, 32.77, 29.61 (2C), 29.09 (2C), 28.68, 27.29 (3C), 25.78, 24.79. EIMS *m*/*z* (%): 314 (M^+). Anal. calcd for $C_{18}H_{31}O_2Cl$: C, 68.65; H, 9.92. Found C, 68.80; H, 10.04.

19-Iodooctadeca-(8*Z*,11*Z*)-dienoic acid (7). A mixture of acid **6** (480 mg, 1.52 mmol) and NaI (686 mg, 4.56 mmol) in dry acetone (6 mL) was stirred at 65°C for 20 h. After the acetone was evaporated, the residue was reconstituted in Et_2O (60 mL). The resulting mixture was washed with H_2O (2×50 mL) and the organic layer was dried over Na_2SO_4. After evaporation under reduced pressure, the crude residue was purified on silica gel (hexane/Et_2O, 1:1) to yield **7** as a colorless oil, which was analyzed to be >98% pure by RP-HPLC; yield: 556 mg (90%). TLC: R_f=0.34 (Et_2O/hexane 1:1). RP-HPLC: RT=4.31 min. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 5.28–5.38 (m, 4H, CH=CH), 3.17 (t, 2H, J=6.8 Hz, 18-CH_2), 2.74 (m, 2H, 10-CH_2), 2.33 (t, 2H, J=7.1 Hz, 2-CH_2), 2.04 (m, 4H, 7-CH_2 and 13-CH_2), 1.80 (m, 2H, 16-CH_2), 1.61 (m, 4H, 3-CH_2 and 17-CH_2), 1.25–1.45 (m, 10H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 177.90, 130.16, 130.05, 128.43, 128.32, 33.76, 30.60, 29.60 (3C), 29.16, 29.09, 28.35, 27.36 (2C), 25.89, 24.86, 7.04. EIMS *m*/*z* (%): 406 (1.39) [M^+], 388 (0.1) [M^+-H_2O], 278 (2.01) [M^+-I+1]. Anal. calcd for $C_{18}H_{31}O_2I$: C, 53.20; H, 7.69. Found C, 53.47; H, 7.41.

19,19-Dimethyleicosa-(8*Z*,11*Z*)-dienoic acid (1a). To a suspension of CuI (234 mg, 1.23 mmol) in THF (7 mL) and HMPA (2 mL), which was cooled to −78°C, *t*-BuLi (1.68 mL, 2.45 mmol) was added with a syringe. After the mixture was stirred for 45 min at −75°C, a solution of acid **7** (154 mg, 0.37 mmol) in THF (2 mL) was added. The resulting mixture was warmed to −50°C and stirred for 1 h. Then the reaction was quenched with sat. aq. NH_4Cl, acidified with HCl (1 M) to pH 5.0 and the organic products were extracted with Et_2O (2×60 mL). The combined etheric extracts were washed with sat. aq. NaCl, dried over Na_2SO_4 and then concentrated under vacuum. Filtration through silica gel (Et_2O/hexane 1:1) and further preparative HPLC (MeOH/CH_3CN/H_2O 47.5:47.5:5) gave 102 mg (82%) of pure **1a**. RP-HPLC: RT=3.38 min. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 5.26–5.36 (m, 4H, CH=CH), 2.74 (m, 2H, 10-CH_2), 2.33 (t, 2H, J=7.3 Hz, 2-CH_2), 2.04 (m, 4H, 7-CH_2 and 13-CH_2), 1.61 (m, 2H, 3-CH_2), 1.25–1.45 (m, 14H), 0.85 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 178.60, 130.26, 129.92, 128.24, 127.93, 44.32, 33.80, 30.51, 29.70, 29.42 (6C), 28.98, 28.89, 27.27, 27.14, 25.65, 24.66, 24.53. EIMS *m*/*z* (%): 336 (3.76) [M^+], 321 (1.5) [M^+-CH_3], 280 (1.89) [$M^+-{}^t$Bu]. Anal. calcd for $C_{22}H_{40}O_2$: C, 78.51; H, 11.98. Found C, 78.79; H, 12.09.

19-Phenylnonadeca-(8*Z*,11*Z*)-dienoic acid (1b). To a suspension of CuI (179 mg, 0.94 mmol) in THF (5 mL) and HMPA (2 mL), which was cooled to −50°C, a solution of $PhCH_2MgCl$ (1.88 mL, 1.88 mmol) was added with a syringe. After the mixture was stirred for 1.5 h at −50°C, a solution of acid **7** (118 mg, 0.29 mmol) in THF (2 mL) was added. The resulting mixture was stirred for 1 h. Then the reaction was quenched with sat. aq. NH_4Cl, acidified with HCl (1 M) to pH 5.0 and the organic products were extracted with Et_2O (2×50 mL). The combined ethereal extracts were washed with sat. aq. NaCl, dried over Na_2SO_4 and then concentrated under vacuum. Filtration through silica gel (Et_2O/hexane 1:1) and further preparative HPLC (MeOH/CH_3CN/H_2O 47.5:47.5:5) gave 85 mg (79%) of pure **1b**. RP-HPLC: RT=2.67 min. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.10–7.25 (m, 5H, Ph), 5.27–5.37 (m, 4H, CH=CH), 2.76 (m, 2H, 10-CH_2), 2.60 (t, 2H, J=8.1 Hz, 19-CH_2), 2.34 (t, 2H, J=7.3 Hz, 2-CH_2), 2.03 (m, 4H, 7-CH_2 and 13-CH_2), 1.60 (m, 4H, 3-CH_2 and 18-CH_2), 1.20-1.40 (m, 14H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 180.48, 143.14, 130.57, 130.24, 129.94, 128.55, 128.33, 125.90, 125.64, 36.22, 34.27, 31.77, 29.86, 29.64 (4C), 29.17 (2C), 27.44 (2C), 25.86, 24.87. EIMS *m*/*z* (%): 370 (12)

$[M^+]$, 352 (0.9) $[M^+-H_2O]$, 279 (2.0) $[M^+-CH_2Ph]$. Anal. calcd for $C_{25}H_{38}O_2$: C, 81.03; H, 10.33. Found C, 79.80; H, 10.54.

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