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Synthesis of (*S*)-(+)-enantiomers of food-relevant (*n*-5)-monoenoic and saturated anteiso-fatty acids by a Wittig reaction

Saskia Thurnhofer and Walter Vetter*

Institute of Food Chemistry, University of Hohenheim, Garbenstr. 28, D-70599 Stuttgart, Germany

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Abstract—We developed an enantioselective synthesis for the food-relevant anteiso-fatty acids a15:0, a16:0, and a17:0. Different (carboxyalkyl)triphenylphosphonium bromide salts were coupled with (*S*)-3-methylpentanal in a Wittig reaction. Mixtures of the obtained *cis*-/*trans*-isomers were separated by Ag^+-HPLC to give the novel *cis*-isomers of (*S*)-(+)-a15:1*n*-5, (*S*)-(+)-a16:1*n*-5, and (*S*)-(+)-a17:1*n*-5. Hydrogenation of the monoenoic products led to (*S*)-(+)-a15:0, (*S*)-(+)-a16:0, and (*S*)-(+)-a17:0, which are essential for the assessment of the bioactivity of tests with standards of anteiso-fatty acids.

1. Introduction

Anteiso-fatty acids (*n*-2-methyl branched fatty acids, aFAs) are found as minor lipid components in a wide range of food (e.g., milk of ruminants, suet, and butterfat) and as major components in the lipids of diverse bacteria.[1–6] At the cellular level, aFAs play an important role in providing an appropriate degree of membrane fluidity at low temperatures, presumably through keeping up the fluid–liquid–crystalline state of membrane lipids.[7–9] The dominating aFAs in food and bacteria are 12-methyltetradecanoic acid (a15:0) and 14-methylhexadecanoic acid (a17:0).[3,10] The contribution of aFAs to the total amount of fatty acids in food ranges from approximately 0 to 3%, which is comparable to levels of conjugated linoleic acids (CLAs) and *trans*-fatty acids.[11,12] Notably, aFAs are chiral, which is in contrast to iso-fatty acids (iFAs). Only little data exist on the enantiomer distribution of aFAs in food. About 0.5% of (*S*)-(+)-a17:0 was determined in ruminant and ewe-milk fat, wool, as well as mutton grease. Comparable amounts of (*S*)-(+)-a15:0 were also detected in ewe-milk fat.[2,13–15] These studies showed that the (*S*)-enantiomers of aFAs are food-relevant.

Next to the saturated aFAs mentioned above, several monoenoic aFAs were detected in different myxobacteria (*Stigmatella aurantiaca* and *Myxococcus xanthus*), some *Bacillus* species (*B. cereus* and *B. xanthus*), *Desulfovibrio desulfuricans*, and also in *Vernix caseosa* and adult human skin lipids.[16–19] Furthermore, a17:1, *cis*-i17:1*n*-5, and *cis*-18:1*n*-5 was found in *B. anthracis*, which belongs to the food-borne bacterial pathogens and aerobic endospore-forming bacilli.[20] Additionally, a15:1 was determined in *Brevibacterium fermentans,* a biotechnological producer of amino acids.[21] In the cases of a15:1 and a17:1, the positions of the double bonds could not be assigned due to the lack of reference standards.

Currently, only a limited number of aFA standards is commercially available, all of which are racemic and saturated. Thus, important experiments on the bioavailability and phase transfer processes could only be performed with racemic aFAs and thus, most likely not under real life conditions. Commonly, FAs are either synthesized by Wittig or Grignard reactions. A Wittig reaction was used to synthesize racemic 12-methylpentadecanoic acid (yield 14%), a15:0 (yield 42%), and *cis*-i17:1*n*-5 (yield 19%).[22–24] In most cases, however, the Grignard pathway was used, which led to yields from 10 to 90%.[25,26] Moreover, (*R*)-12-methylhexadecanoic acid (yield 74%) and (*S*)-aFAs were recently obtained in a total yield of 10%.[27,28]

The goal of this study was to produce both saturated and monoenoic aFAs as racemates and as pure (*S*)-enantiomers. For this reason, we performed the C–C-coupling by a Wittig reaction, which predominantly leads to *cis*-monoenoic aFAs. Aliquots of monoenoic aFAs can be collected for analytical purposes whereas the remaining part can be converted into the corresponding saturated enantiopure (*S*)-aFAs by hydrogenation.

Keywords: Methyl branched fatty acids; Anteiso-fatty acids; Monoenoic anteiso-fatty acids; Wittig reaction; Enantioselective synthesis; Silver-ion chromatography.
* Corresponding author. Tel.: +49 711 459 24016; fax: +49 711 459 24377; e-mail: w-vetter@uni-hohenheim.de

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2. Results and discussion

Production of monoenoic aFAs **6a–c** via a Wittig reaction required ω-bromocarboxylic acids and a racemic or enantiopure methyl branched aldehyde (Fig. 1). For enantioselective synthesis, the methyl branch of the aldehyde must not be in α-position in order to prevent racemization via the corresponding enol. Hence, the shortest appropriate aldehyde was 3-methylpentanal, which was also selected since the required substrates for the preparation of a15:0–a17:0 (**1a–c**) were commercially available (Fig. 1). However, both racemic and (*S*)-3-methylpentanal **5** were not available and were prepared from racemic and (*S*)-(+)-3-methylpentanol **4** by mild oxidation using Dess–Martin periodinane (DMP) (Fig. 1).[29] Impurities of 3-methylpentanol in 3-methylpentanal on the basis of GC/EI-MS were <2%. The spectroscopic data[30] and the specific rotation of enantiopure **5** matched those published for the compound ($[\alpha]_D^{20}$ −4.00 and −2.46).[31,32] The resulting 3-methylpentanal was used without any purification step for the subsequent Wittig reaction (see below).

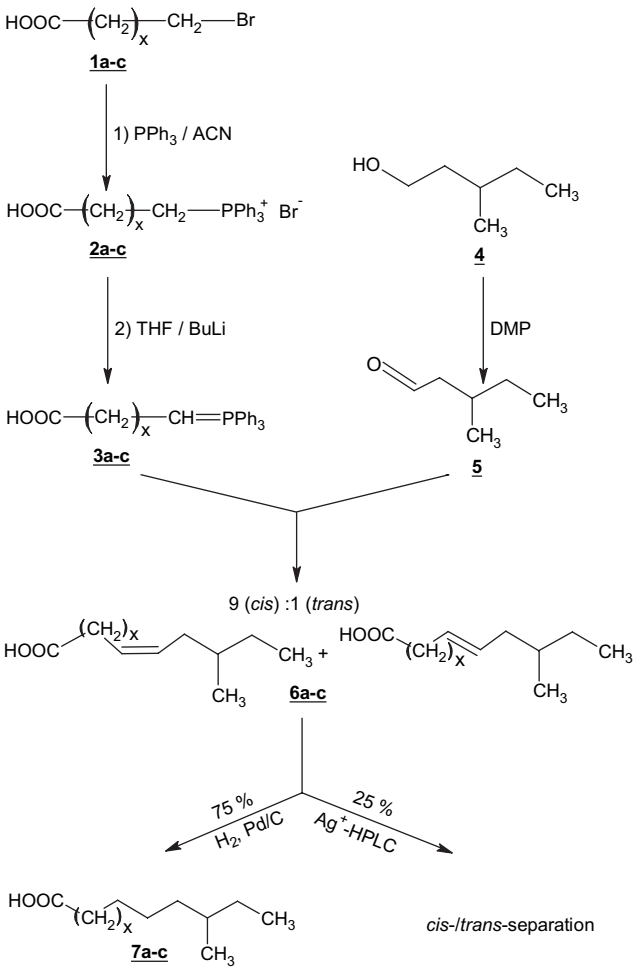

Figure 1. Synthesis pathway for food-relevant monoenoic and saturated anteiso-fatty acids via a Wittig-coupling. ω-Bromoalkylcarboxylic acids **1** (**a**: *x*=7, **b**: *x*=8 and for **c**: *x*=9) were transferred into the corresponding triphenylphosphonium bromide salts **2a–c**. Addition of either racemic or (*S*)-3-methylpentanal **5** obtained from (*S*)-3-methylpentanol **4** leads to monoenoic aFAs **6a–c**. Aliquots (25%) of *cis*-/*trans*-isomers of **6a–c** were separated by Ag^+-HPLC whereas hydrogenation of the bulk of **6a–c** resulted in the corresponding saturated aFAs **7a–c**.

In the first step of the Wittig reaction, **1a–c** were reacted with equimolar amounts of triphenylphosphine to obtain the corresponding triphenylphosphonium bromide salts **2a–c** (Fig. 1). When acetonitrile (ACN) was used as solvent, the resulting one-phase system provided better yields of **2a–c** compared to the two-phase system with toluene initially used. This was most likely due to thorough mixing of the solution during the entire course of reaction. After formation of **2a–c** in ACN, an additional purification step with toluene by heating under reflux was carried out in order to remove the remaining educts. Subsequent treatment with anhydrous diethyl ether converted the oils into white salts **2a–c**. Even **2a** was obtained as a nearly white powder, which is in contrast to reports in literature, where only moisture- and air-sensitive glasses could be obtained.[33,34] Purified **2a–c** were hygroscopic and had to be redried directly prior the Wittig-coupling. As a consequence of all efforts, the yields (91–97%) of **2a–c** were higher than reported in literature (62–75%).[2,34,35] The spectral data of the synthesized triphenylphosphonium bromide salts (**2a–c**) were in agreement with those reported in literature.[34,35]

The Wittig reaction (performed at 0–5 °C) led to 88.6–91.1% *cis*- and 8.9–11.4% *trans*-isomers of aFAs **6a–c**, which were obtained as weak yellow oils. After separation of impurities (triphenylphosphine oxide and byproducts), the yields of **6a–c** of this step were 36.1–47.0% and the total yields (including all steps) were 25.0–41.4% (Table 1). The yields correlated with the chain length of the triphenylphosphonium bromide salts, which is most likely owing to the higher hygroscopy of the shorter ones. Additionally, ^{1}H NMR data confirmed the identity of **6a–c**, which was comparable with the reported chemical shifts for the structurally similar 17-methyl-6(*cis*)-octadecenoic acid methyl ester.[36]

Aliquots of the monoenoic aFAs **6a–c** were treated with BF_3/MeOH to produce fatty acid methyl esters (FAMEs). The resulting *trans*- (first eluted) and *cis*-isomers (second eluted, $\Delta RRI_{16:0\ Me}$=0.0183–0.0288) of monoenoic aFAs showed identical GC/EI-mass spectra (Fig. 2). The methyl esters of *cis*- and *trans*-monoenoic aFAs showed the expected molecular ion at low abundance, the base peak at *m/z* 55, and further characteristic fragment ions at $[M-61]^+$ and $[M-79]^+$ (Fig. 2).[15,37] Furthermore, the GC retention times were in the expected range.

Aliquots of the monoenoic (*S*)-aFAs **6a–c** were methylated and subjected to silver-ion high-performance liquid chromatography (Ag^+-HPLC) for the separation of *cis*- and *trans*-isomers.[38] After this procedure neat standards of both *cis*- and *trans*-isomers of **6a–c** were obtained (Table 1).

Saturated aFAs **7a–c** were finally obtained by hydrogenation of aFAs **6a–c** (Fig. 2). The mass spectra and relative retention indices (Table 1) of the synthesized methyl esters of enantiopure aFAs **7a–c** were identical with corresponding data of the commercially available racemates (data not shown). Additionally, the mass spectra of saturated FAME showed the typical and major fragment ions *m/z* 74 and *m/z* 87, which contribute at >50% to the total ion current of saturated fatty acids.[39,40] Slight dextrorotation was observed for the methyl esters of (*S*)-(+)-a15:0 and (*S*)-(+)-a17:0, which was similar to those reported for the free fatty acids of (*S*)-(+)-a15:0 and (*S*)-(+)-a17:0 (Table 1).[41,42] Slight

Table 1. Relative retention indices (RRI), direction of rotation, and total yields of *cis*- and *trans*-monoenoic aFAs and the corresponding saturated (*S*)-(+)-aFAs

Product	RRI[a]	$[\alpha]_D^{20}$, this study	$[\alpha]_D^{20}$, literature data	Total yields[b] [%]
Monoenoic aFAs				
6a *cis*-(*S*)-a15:1*n*-5 ME	0.9712	+4.65 (*c* 1.6 g/100 mL) $CHCl_3$	—	25.0
6a *trans*-(*S*)-a15:1*n*-5 ME	0.9529			
6b *cis*-(*S*)-a16:1*n*-5 ME	1.0298	+4.31 (*c* 1.9 g/100 mL) $CHCl_3$	—	27.0
6b *trans*-(*S*)-a16:1*n*-5 ME	1.0105			
6c *cis*-(*S*)-a17:1*n*-5 ME	1.1016	+3.82 (*c* 1.7 g/100 mL) $CHCl_3$	—	41.4
6c *trans*-(*S*)-a17:1*n*-5 ME	1.0728			
Saturated aFAs				
7a (*S*)-a15:0 ME	0.9277	+5.56[a] (*c* 3.6 g/100 mL) $CHCl_3$	+5.07[41] (*c* 2.08 g/100 mL) $CHCl_3$	25.0
7b (*S*)-a16:0 ME	0.9822	+5.17[a] (*c* 2.9 g/100 mL) $CHCl_3$	+3.3[28] (*c* 1.73 g/100 mL) $CHCl_3$	27.0
7c (*S*)-a17:0 ME	1.0440	+4.29[a] (*c* 3.5 g/100 mL) $CHCl_3$	+4.49[22,42] neat	41.4

[a] Retention indices of aFAMEs relative to 16:0 ME (RRI=1.0000).
[b] Including all steps.

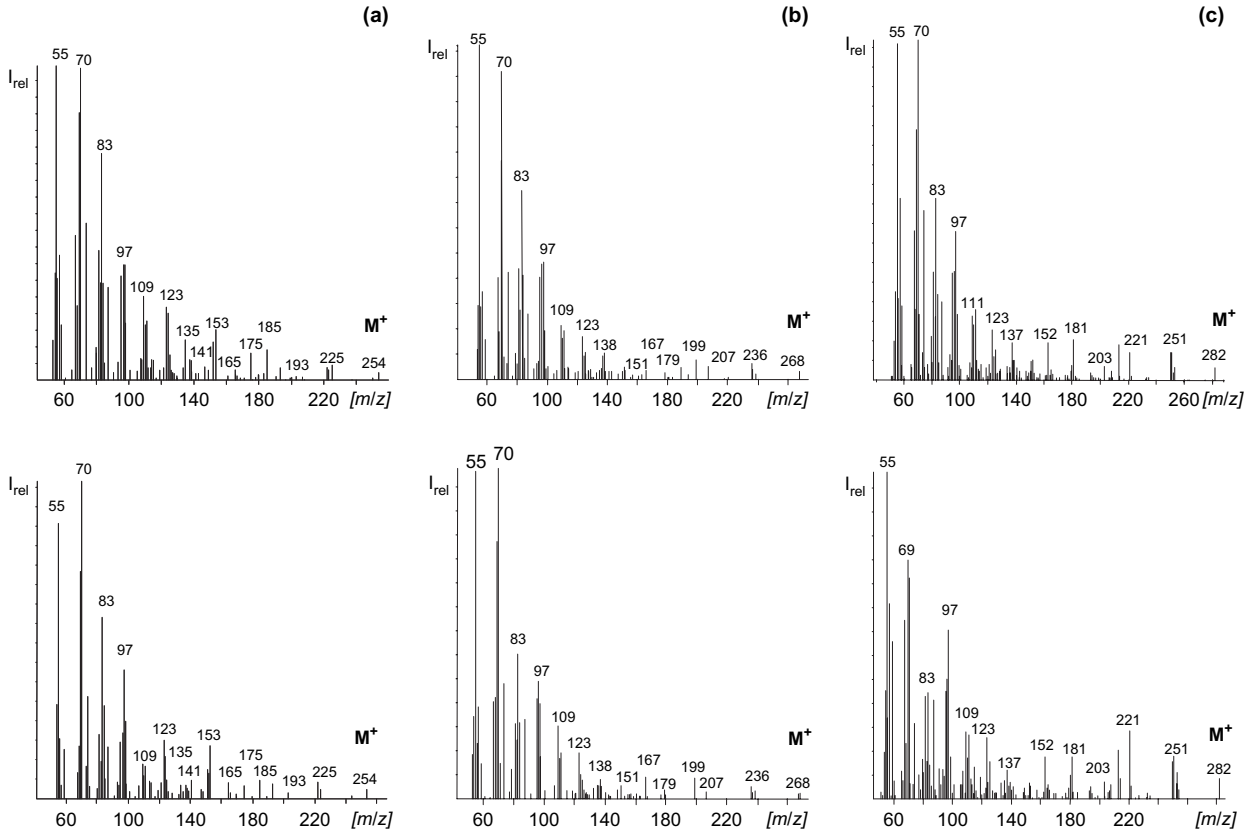

Figure 2. GC/EI-mass spectra of the methyl esters of *cis*- (top) and *trans*-monoenoic aFAs (bottom) of (a) a15:1*n*-5, (b) a16:1*n*-5, and (c) a17:1*n*-5.

clockwise rotation was also found for (*S*)-(+)-a16:0 methyl ester. The specific optical rotation of the novel monoenoic aFAs showed slightly lower dextrorotation compared to the saturated aFAs as methyl esters and free fatty acids.

The performed synthesis pathway is suitable for the production of food-relevant aFAs. Both saturated and (*n*-5)-*cis*-monoenoic (~90%) and (*n*-5)-*trans*-monoenoic forms (~10%) were obtained as pure standards.

3. Conclusion

The synthesized (*S*)-(+)-enantiomers of a15:0, a16:0, and a17:0 can be used in diverse studies to assess the significance of enantioselectivity of aFAs in biological systems. In addition to the saturated aFAs, our synthesis led for the first time to enantiopure and racemic (*n*-5)-monoenoic aFAs. Recently, 16:1*n*-5 and 14:1*n*-5 and further fatty acids with double bonds between (*n*-7) and (*n*-4) were identified in myxobacteria.[19] As was mentioned above, a15:1 and a17:1 have been identified in food-related bacteria, but the positions of the double bonds have not yet been determined.[20,21] The monoenoic aFAs synthesized in this study can be used for a first screening of food samples for these compounds along with unequivocal structure confirmation. Biosynthesis of minute amounts of monoenoic aFAs has been linked with bacterial desaturases.[19] Therefore, these monoenoic aFAs may be suitable markers for the (previous) presence of bacteria in food.

4. Experimental

4.1. General

Melting points were determined with a Büchi B-545 instrument and are uncorrected. ^{1}H NMR spectra were recorded with a Varian Inova 300 MHz instrument in 5-mm tubes (5–20 mg samples in 700 μL $CDCl_3$). IR spectral data were recorded with a Nicolet Avata 320 FT-IR spectrometer. Hydrogenation was performed with an Anton Paar Synthos 3000 apparatus. Optical rotations were measured with a Perkin Elmer Model 341 digital polarimeter equipped with a thermostat attuned to 20 °C.

4.2. Materials and chemicals

9-Bromononanoic acid (99%) was purchased from TCI (Zwijndrecht, Belgium). 10-Bromodecanoic acid and 11-bromoundecanoic acid were obtained from Aldrich (both 99% purity, Taufkirchen, Germany). Triphenylphosphine, *n*-butyllithium solution (2.5 M in *n*-hexane), sodium in pieces, benzophenone, and anhydrous sodium sulfate were from Fluka (Taufkirchen, Germany). (*S*)-(+)-3-Methylpentanol (>98% purity) and racemic 3-methylpentanol (purity >99%) were from TCI. Dess–Martin periodinane (DMP, 97% purity) was from Aldrich. Boron-trifluoride–methanol-complex solution (13–15% BF_3 in methanol) was from Riedel-de-Haën (Seelze, Germany). Toluene, tetrahydrofuran, and diethyl ether were from Roth (Karlsruhe, Germany). *n*-Hexane (HPLC grade) and acetonitrile (HPLC grade) were from Fisher Scientific (Schwerte, Germany). All solvents used for the reactions were freshly dried over sodium or molecular sieves and distilled.

4.3. Conversion of aFAs into aFAMEs

aFAs (4 mg) and 0.5 mL methanolic KOH (0.5 M) were heated for 5 min at 80 °C. After cooling, 1 mL methanolic BF_3-solution was added and heated for additional 5 min at 80 °C. Then, 2 mL saturated sodium chloride solution and 2 mL *n*-hexane were added to the cooled solution (ice bath). The hexane-phase containing the aFAMEs was separated and analyzed by GC/EI-MS.[43]

4.4. Gas chromatography with electron ionization mass spectrometry (GC/EI-MS)

A Hewlett–Packard 5890 series II gas chromatograph was used in combination with a 5971A mass selective detector. Sample solution (1 μL) was injected with a 7673A autosampler (splitless mode, split opened after 2 min). The injector and transfer line temperatures were kept at 250 °C and 280 °C. Analyses were performed on a 60 m×0.25 mm i.d. fused-silica capillary column (CP Sil 88, Supelco, Taufkirchen, Germany). The carrier gas helium (purity 5.0) was used at a constant flow rate of 1 mL/min. The GC oven program started at 60 °C (hold time 1 min) was then raised at 7 °C/min to 180 °C (hold time 2 min), then at 3 °C/min to 200 °C (hold time 2 min), and finally at 3 °C/min to 220 °C (hold time 10 min). Mass spectra (*m/z* 50–450) were recorded at a rate of five scans per second with an ionization energy of 70 eV. The temperature of the ion source was 170 °C.

4.5. Synthesis of aFAs

4.5.1. Preparation of 2a–c. Triphenylphosphine (5.1 g, 0.019 mmol) was dissolved in 20 mL of freshly dried ACN in a 100 mL round-bottomed flask. An equimolar amount of ω-bromoalkylcarboxylic acid **1a–c** in 20 mL of ACN was slowly added to the stirred triphenylphosphine solution. The reagents readily dissolved and the orange colored reaction mixture was heated under reflux for 48 h at 90 °C under argon. The solvent of the reaction mixture was then evaporated and the (carboxyalkyl)triphenylphosphonium bromide salts **2a–c** were obtained as slightly yellow oils, which proved insoluble in toluene (Fig. 1).[44]

4.5.2. Purification and isolation of 2a–c. Toluene (20 mL) was added to each slight yellow oil and heated under reflux for 30 min. The toluene phase was decanted and the cleaning process was repeated with 20 mL of toluene. Afterwards, 30 mL of anhydrous diethyl ether was added to the oily residue and recrystallization in the fridge resulted in a slightly yellow solid. The obtained solid was triturated with diethyl ether and the precipitate was filtered off and washed several times with anhydrous diethyl ether. Drying overnight in a vacuum centrifuge produced white powders of **2a–c** in 91–99% yields. Analytical data of **2a** (8-carboxyoctyl)-triphenylphosphonium bromide: yield 91%; melting point 114–115 °C; IR spectral data (CH_2Cl_2, cm^{-1}): 2925, 2850, 1710, 1435, 1110, 750, 720, 690; ^{1}H NMR ($CDCl_3$, ppm): 7.70–7.86 (m, 15H); 3.71 (m, 2H); 2.41 (t, 2H); 1.30–1.62 (m, 12H). **2b** (9-Carboxynonyl)triphenylphosphonium bromide: yield 95%; melting point 93–99 °C; IR spectral data (CH_2Cl_2, cm^{-1}): 2925, 2850, 1715, 1435, 1110, 750, 720, 685; ^{1}H NMR ($CDCl_3$, ppm): 7.75–7.89 (m, 15H); 3.73 (m, 2H); 2.42 (t, 2H); 1.27–1.60 (m, 14H). **2c** (10-Carboxydecyl)triphenylphosphonium bromide: yield 97%; melting point 104–106 °C; IR spectral data (CH_2Cl_2, cm^{-1}): 2920, 2850, 1710, 1435, 1110, 750, 725, 690; ^{1}H NMR ($CDCl_3$, ppm): 7.75–7.89 (m, 15H); 3.75 (m, 2H); 2.42 (t, 2H); 1.29–1.64 (m, 16H).

4.5.3. Oxidation of 3-methylpentanol 4 to 3-methylpentanal 5. Dess–Martin periodinane (3.52 g, 8.29 mmol) was dissolved in 10 mL of anhydrous methylene chloride. Under stirring, a solution of 3-methylpentanol **4** (1 mL, 8.06 mmol) in 10 mL of anhydrous methylene chloride was added dropwise (Fig. 1). The solution was kept for 1 h at room temperature. Diethyl ether (50 mL) was added, and the reaction mixture was transferred to a separating funnel. Then, 50 mL of a 1:1 (v/v) mixture of aqueous 1 M sodium thiosulfate and a solution of saturated sodium hydrogencarbonate was added. After 10 min of vigorous shaking, the organic phase was separated and washed with 50 mL of saturated sodium hydrogencarbonate solution and 50 mL of distilled water. The organic phase was dried over anhydrous sodium sulfate, and the organic solvent was carefully removed by microdistillation.[29] (*S*)-(−)-3-Methylpentanal was obtained in the above-mentioned way by using (*S*)-(+)-3-methylpentanol. The yield was 98%. The identity and purity of the obtained (*S*)-(−)-3-methylpentanal **5** were proved by GC/EI-MS, IR, and ^{1}H NMR.[30] Analytical data of **5**: IR spectral data (CH_2Cl_2, cm^{-1}): 2960, 2930, 2880, 1720. GC/EI-MS (*m/z*): $[M]^+$ 100(1), 82(3), 71(18), 56(100), 43(48), 41(73), 29(42), 15(4); ^{1}H NMR ($CDCl_3$, ppm): 9.80 (s, 1H), 2.40 (m, 2H),

1.50–1.00 (m, 9H). Optical rotation $[\alpha]_D^{20}$ −2.6 (*c* 2.1 g/100 mL in CH_2Cl_2).

4.5.4. Wittig-coupling of the aldehyde 5 with 2a–c. Each (carboxyalkyl)triphenylphosphonium bromide salt **2a–c** (1.0 mmol) was dissolved in 100 mL of freshly distilled tetrahydrofuran (THF). The solution was cooled to 0–5 °C. Then a solution of *n*-BuLi (2.5 M in *n*-hexane, 0.63 mmol) in 5 mL THF was added dropwise to produce the bright orange ylide **3** within 20 min at 0–5 °C.[45] 3-Methylpentanal or (*S*)-(−)-3-methylpentanal **5** (1.0 mmol), dissolved in 5 mL of THF, was added dropwise and the solution was stirred for 4 h at room temperature. Afterwards, the mixture was poured on ice and acidified with 1 M HCl. The aqueous solution was extracted several times with 20 mL of diethyl ether and the combined organic phases were washed twice with saturated sodium chloride solution. The organic phase was dried over anhydrous sodium sulfate and filtered. After removal of the solvent, the monoenoic aFAs **6a–c** were obtained as weak yellow oils. For purification, ~60% of the impure oils of aFAs **6a–c** were eluted from silica gel with petroleum ether–ethyl acetate (3:1; v/v). 54.0 mg (36% yield) of aFA **6a**, 51.0 mg (33.8% yield) of aFA **6b**, and 70.5 mg (47.0% yield) of aFA **6c** were obtained as colorless oils. The resulting mixtures of *cis*- and *trans*-isomers contained 88.6% (**6a**), 90.4% (**6b**), and 91.1% (**6c**) of the *cis*-isomer.

^{1}H NMR ($CDCl_3$, ppm): (*S*)-(+)-**6a**: 5.43–5.37 (m, 2H), 2.40–2.35 (t, 2H), 2.10–2.00 (m, 4H), 1.96–1.86 (m, 2H), 1.69–1.60 (m, 2H), 1.47–1.23 (m, 8H), 1.20–1.17 (m, 1H), 0.99–0.88 (m, 6H). (*S*)-(+)-**6b**: 5.45–5.37 (m, 2H), 2.41–2.36 (t, 2H), 2.10–2.02 (m, 4H), 1.94–1.85 (m, 2H), 1.69–1.62 (m, 2H), 1.48–1.23 (m, 10H), 1.20–1.13 (m, 1H), 0.99–0.89 (m, 6H). (*S*)-(+)-**6c**: 5.44–5.38 (m, 2H), 2.41–2.36 (t, 2H), 2.10–2.02 (m, 4H), 1.94–1.87 (m, 2H), 1.72–1.65 (m, 2H), 1.47–1.23 (m, 12H), 1.20–1.15 (m, 1H), 0.99–0.89 (m, 6H); ^{1}H NMR data of racemic and enantiopure *cis*-/*trans*-monoenoic aFAs were identical. Despite the dominance of *cis*-isomers, the presence of ~10% of *trans*-isomers caused a slight downfield shift (~0.02–0.03 ppm) of the olefinic protons in the ^{1}H NMR spectra. Thus, NMR data is only reported for the neat *cis*-monoenoic aFAs obtained after separation of the *trans*-isomers (Section 4.5.5).

4.5.5. Separation of *cis*- and *trans*-monoenoic aFA 6a–c by silver-ion high-performance liquid chromatography (Ag^+-HPLC). Ag^+-HPLC analyses were performed using a Varian solvent pump equipped with a Rheodyne 7010 injector fitted with a 100 µL loop and a Varian ProStar 325 UV–vis detector with dual wavelength mode set at 206 and 234 nm. After converting the monoenoic aFAs **6a–c** into fatty acid methyl esters (FAMEs), 100 µg of *cis*- and *trans*-monoenoic aFAs in 50 µL *n*-hexane were injected and separated using a ChromSpher 5 Lipids column (250 mm×4.6 mm i.d. stainlees steel, a silver-modified cation exchange ligand-covered 5 µm spherical silica column; Varian, Darmstadt, Germany). An isocratic solvent system of *n*-hexane with 0.08% ACN was used as mobile phase at a flow rate of 1.0 mL/min.[38] The fractions containing the *cis*- and *trans*-monoenoic aFAs **6a–c** were collected with a 701 fraction collector (Varian) and analyzed by GC/EI-MS. The fractionation was repeated several times and the corresponding fractions were pooled to obtain sufficient material for further analyses. Analytical data of *cis*-(*S*)-(+)-**6a** ME: GC/EI-MS (*m*/*z*): $[M]^+$ 254(2.0), $[M-61]^+$ 193(5.2), $[M-79]^+$ 175(3.3), 153(14.2), 55(100); ^{1}H NMR ($CDCl_3$, ppm): 5.41–5.34 (m, 2H), 3.68 (s, 3H), 2.34–2.29 (t, 2H), 2.06–1.99 (m, 4H), 1.92–1.85 (m, 2H), 1.65–1.60 (m, 2H), 1.44–1.20 (m, 8H), 1.17–1.13 (m, 1H), 0.91–0.86 (m, 6H). *trans*-(*S*)-(+)-**6a** ME: GC/EI-MS (*m*/*z*): $[M]^+$ 254(2.4), $[M-61]^+$ 193(5.0), $[M-79]^+$ 175(1), 153(11.7), 55(100). *cis*-(*S*)-(+)-**6b** ME: GC/EI-MS (*m*/*z*): $[M]^+$ 268(3.5), $[M-61]^+$ 207(7.1), $[M-79]^+$ 189(5.7), 167(15.7), 55(100); ^{1}H NMR ($CDCl_3$, ppm): 5.41–5.33 (m, 2H), 3.68 (s, 3H), 2.34–2.29 (t, 2H), 2.06–1.99 (m, 4H), 1.92–1.85 (m, 2H), 1.65–1.60 (m, 2H), 1.44–1.20 (m, 8H), 1.17–1.13 (m, 1H), 0.91–0.86 (m, 6H). *trans*-(*S*)-(+)-**6b** ME: GC/EI-MS (*m*/*z*): $[M]^+$ 268(3.9), $[M-61]^+$ 207(3.9), $[M-79]^+$ 189(3.4), 167(9.4), 55(100). *cis*-(*S*)-(+)-**6c** ME: GC/EI-MS (*m*/*z*): $[M]^+$ 282(3.4), $[M-61]^+$ 221(6.8), $[M-79]^+$ 203(4.6), 181(12.2), 55(100); ^{1}H NMR ($CDCl_3$, ppm): 5.41–5.34 (m, 2H), 3.68 (s, 3H), 2.34–2.29 (t, 2H), 2.06–1.99 (m, 4H), 1.92–1.85 (m, 2H), 1.65–1.60 (m, 2H), 1.44–1.20 (m, 8H), 1.18–1.13 (m, 1H), 0.91–0.86 (m, 6H). *trans*-(*S*)-(+)-**6c** ME: GC/EI-MS (*m*/*z*): $[M]^+$ 282(2.0), $[M-61]^+$ 221(5.3), $[M-79]^+$ 203(6.3), 181(9.5), 55(100). The ^{1}H NMR data of racemic and enantiopure *cis*-monoenoic aFAs were identical.

4.5.6. Catalytic hydrogenation of the monoenoic aFAs 6a–c to the saturated aFAs 7a–c. The saturated aFAs **7a–c** were prepared by catalytic hydrogenation of 30 mg of monoenoic aFAs **6a–c** in 30 mL of absolute methanol containing 10% palladium on charcoal (10% Pd/C). The suspension was stirred for 30 min in an H_2 atmosphere (4.8 bar).[18,21] The hydrogenated product was filtered through Celite 545 to remove the catalyst. The resulting solution was concentrated and analyzed by GC/EI-MS after esterification. Approximately 30 mg **7a–c** corresponding to 100% yield were obtained (Table 1). IR spectral data ($CHCl_3$, cm^{-1}): (*S*)-(+)-**7a–c** ME: 2927, 2854, 1732. (*S*)-(+)-**7a** ME: GC/EI-MS (*m*/*z*): $[M]^+$ 256(1.3), $[M-29]^+$ 227(1.1), $[M-43]^+$ 213(4.5), 87(58.8), 74(100); ^{1}H NMR ($CDCl_3$, ppm): 3.68 (s, 3H), 2.34–2.29 (t, 2H), 1.63–1.60 (m, 2H), 1.44–1.27 (m, 18H), 1.15–1.10 (m, 1H), 0.89–0.84 (m, 6H). (*S*)-(+)-**7b** ME: GC/EI-MS (*m*/*z*): $[M]^+$ 270(3.4), $[M-29]^+$ 241(1.2), $[M-43]^+$ 227(3.4), 87(58.8), 74(100); ^{1}H NMR ($CDCl_3$, ppm): 3.68 (s, 3H), 2.34–2.29 (t, 2H), 1.65–1.60 (m, 2H), 1.44–1.27 (m, 20H), 1.17–1.10 (m, 1H), 0.89–0.84 (m, 6H). (*S*)-(+)-**7c** ME: GC/EI-MS (*m*/*z*): $[M]^+$ 284(3.7), $[M-29]^+$ 255(1.5), $[M-43]^+$ 241(4), 87(58.9), 74(100); ^{1}H NMR ($CDCl_3$, ppm): 3.68 (s, 3H), 2.34–2.29 (t, 2H), 1.65–1.60 (m, 2H), 1.44–1.27 (m, 22H), 1.15–1.10 (m, 1H), 0.89–0.84 (m, 6H). The ^{1}H NMR data of racemic and enantiopure aFAs **7a–c** were identical and congruent with the commercially available racemic standards.

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