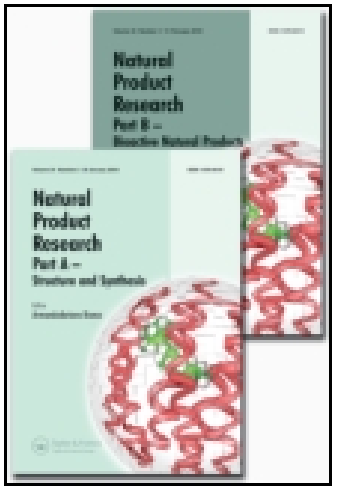

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Synthesis of 1-octacosanol and GC-C-IRMS discrimination of samples from different origin

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Synthesis of 1-octacosanol and GC-C-IRMS discrimination of samples from different origin

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Lately, long-chain primary alcohols have been investigated in depth on account of their biological activities. In particular, 1-octacosanol ($C_{28}H_{57}OH$), the main component of policosanol, the hypolipidaemic fatty alcohol mixture obtained from sugar cane wax, has been the subject of a multitude of pharmacological studies. The aim of this work was to search a convenient synthetic protocol for the preparation of 1-octacosanol in a gram scale. The key step was a Wittig reaction between the octadecyltriphenylphosphonium ylide and the methyl 10-oxodecanoate. Some steps were further improved by power ultrasound and microwave irradiation, either alone or in combination. Our methodology is suitable for a rapid generation of homologues by varying the chain length in the alkyl halide. Due to the high commercial value, a series of 1-octacosanol samples, either isolated from natural sources or from synthesis (different origin and suppliers), were analysed by gas chromatography-combustion-isotopic ratio mass spectrometry (GC-C-IRMS) and according to the carbon isotopic content, classified on the basis of their origin.

Keywords: octacosanol; synthesis; GC-C-IRMS; ultrasound; microwaves

1. Introduction

Since the 1980s, higher aliphatic primary alcohols have been a subject of intensive research. Early work was mainly focused on the biological activities of 1-octacosanol ($C_{28}H_{57}OH$) and 1-triacontanol ($C_{30}H_{61}OH$), isolated from germ oils and beeswax (Houtz, Ries, & Tolbert, 1985; Norris, Denys, & Fallat, 1986; Saint-John & McNaughton, 1986; Sho, Chinen, & Fukuda, 1984). It was reported that the addition of sugarcane wax to the diet lowered the levels of plasma lipids in the rat, although the authors did not attribute the effect to long-chain alcohols (Sho et al., 1984). Later on, when the effects of 1-octacosanol on lipid metabolism were investigated in mice submitted to exercise training, a reduction of hepatic lipid (mainly triglycerides) was observed (Shimura, Hasegawa, Takano, & Suzuki, 1987).

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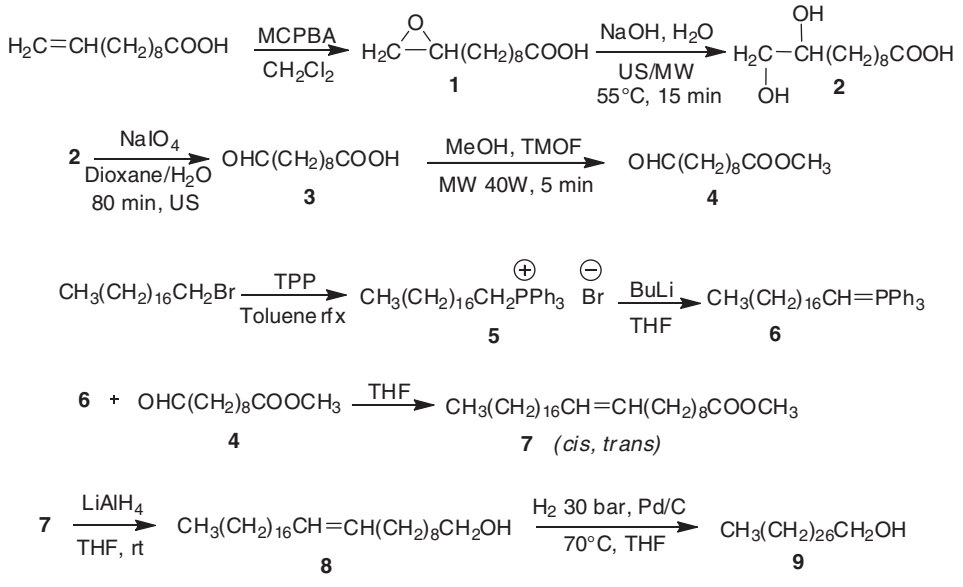
It was only from the 1990s that a spate of results appeared, documenting the cholesterol-lowering, anti-platelet and antioxidant effects of a substance named policosanol (Hernández et al., 1992; Más, 2000). Policosanol was described by Cuban researchers as a mixture of eight higher aliphatic primary alcohols obtained from the wax of sugarcane (*Saccharum officinarum* L.). It contained: 1-tetracosanol (C_{24}), 1-hexacosanol (C_{26}), 1-heptacosanol (C_{27}), 1-octacosanol (C_{28}) – the most abundant (60–70%), 1-nonacosanol (C_{29}), 1-triacontanol (C_{30}), 1-dotriacontanol (C_{32}) and 1-tetratriacontanol (C_{34}). The relative abundance of these alcohols and their total content defines the identity of policosanol. These mixtures are easily analysed by gas chromatography (GC) and GC coupled with mass spectrometry (GC-MS) (Sierra, González, & Magraner, 2002). Although more work is needed for a definitive conclusion to be reached, the overwhelming majority of clinical investigations corroborate the dose-dependent efficacy of original policosanol as an anti-lipidaemic agent (Viola, Oliaro, Binello, & Cravotto, 2008). Pure octacosanol has been investigated as a possible treatment for Parkinsonism and amyotrophic lateral sclerosis (Norris et al., 1986; Snider, 1984). It has also been used by athletes to enhance their performance (Beltz & Doering, 1993; Voy, 1986; Woolley, 1991). Most of the studies related to the pharmacokinetics and metabolism of octacosanol in human and animal subjects were performed using radioactively labelled octacosanol (Kabir & Kimura, 1993, 1994, 1995; Menéndez et al. 1996). A direct absorption of the fatty alcohol was shown in the mucosa cells without complete oxidation to the corresponding fatty acid metabolite. More recently, a clinical study conducted by Keller, Gimmler and Jahreis (2008) demonstrated that octacosanol decreases in the concentration of faecal cholesterol end products that should be derived from a systemic effect on cholesterol metabolism, even though the serum cholesterol levels were not influenced. Octacosanol is commercially available and it can be obtained by isolation from natural sources, i.e. by molecular distillation under a high vacuum (Chen et al., 2007) or by synthesis (Cravotto, 2005). The present article describes a new efficient method of synthesis of 1-octacosanol and a way to easily discriminate samples coming from different natural sources, place of production, manufacturing protocol or starting materials for the synthesis by means of isotopic ratio mass spectrometer (IRMS).

2. Results and discussion

The present article describes an efficient synthetic procedure to obtain 1-octacosanol in gram scale. Some critical steps were improved by irradiation with power ultrasound and/or microwaves, alone or combined. In particular, the formation of the diol by epoxide opening, as described in a previous paper (Palmisano et al., 2007), was rapidly achieved by simultaneous irradiation (Scheme 1). Undecylenic acid and stearyl bromide are commercially available cheap starting materials that make the synthesis very attractive for industrial application (Cravotto, 2005). All the steps promoted by US or MW irradiation may also be performed under conventional conditions, though with slightly lower yields and longer reaction times. The key step was a Wittig reaction between an octadecyltriphenyl phosphonium ylide and a methyl 10-oxodecanoate, the two building blocks of the long-chain monofunctionalised target molecule. In agreement with a flow-chart study of our synthetic



Scheme 1. Synthesis of 1-octacosanol.

process, it emerged that procedure and costs were compatible with an industrial scale up.

Direct gas chromatography-combustion-isotopic ratio mass spectrometry (GC-C-IRMS) analysis, without any derivatisation of the samples, afforded $\delta^{13}C$ data reported in Table 1 with standard deviations. ^{13}C data of octacosanol samples were ranging in a wide interval, between −16 and −36‰. Each value resulted as an average of four runs (SD < 2%). All δ ^{13}C data versus PDB are plotted with error bars in Figure 1.

Octacosanol samples prepared by synthesis in two different laboratories (Batch 1 and 2) using starting materials from different suppliers showed ^{13}C deviations close to each other, but with significantly different mean values: −29.7‰ for Batch 1 and −32.5‰ for Batch 2.

The $\delta^{13}C$ values of the industrial samples vary (−29‰ for Sigma-Aldrich and higher for Giellepi, up to −17‰), proving a different origin of the starting material. In fact, octacosanol samples provided by Giellepi showed that ^{13}C figures are not significantly different from natural Cuban samples (Dalmer sa.), conclusive evidence for the same natural source (i.e. sugarcane wax) of the samples. 1-Octacosanol can be isolated from the wax of sugarcane (*Saccharum officinarum,* L.) after hydrolysis and purification. Samples have a content of 1-octacosanol ranging from 70 to 95%. Under our GC experimental conditions, the C_{28} alcohol was easily separated from other fatty alcohols present in low amounts (mainly C_{26} and C_{30}). Surprisingly, Indian octacosanol samples of natural origin (claimed to be from sugar cane wax) showed a considerably different content of ^{13}C compared to the Cuban ones. This fact is supporting evidence for a rather different pedo-climatic environment in which the plants grew. Standard deviations are lower than 0.5% for industrial, natural samples and the product coming from Batch 1; octacosanol samples from Batch 2 showed less precise figures, with standard deviation values around 1%.

Table 1. δ ^{13}C values with standard deviations for octacosanol samples from industrial, natural and synthetic sources.

Sample identifier	Origin of sample[a]		δ^{13}C vs. PDB
1	Sigma–Aldrich	I	(−29.24 ± 0.04)
2	Sigma–Aldrich	I	(−29.04 ± 0.03)
3	Sigma–Aldrich	I	(−29.05 ± 0.06)
4	Sigma–Aldrich	I	(−29.09 ± 0.06)
5	Sigma–Aldrich	I	(−29.46 ± 0.07)
6	Sigma–Aldrich	I	(−29.38 ± 0.09)
7	Sigma–Aldrich	I	(−29.46 ± 0.33)
8	Sigma–Aldrich	I	(−29.21 ± 0.18)
9	Giellepi	I	(−16.76 ± 0.02)
10	Giellepi	I	(−16.70 ± 0.09)
11	Giellepi	I	(−17.08 ± 0.17)
12	Giellepi	I	(−16.96 ± 0.17)
13	Giellepi	I	(−16.84 ± 0.16)
14	Giellepi	I	(−16.87 ± 0.14)
15	Giellepi	I	(−17.13 ± 0.15)
16	Giellepi	I	(−17.13 ± 0.15)
17	SCW (Cuba)	N	(−17.06 ± 0.02)
18	SCW (Cuba)	N	(−16.90 ± 0.08)
19	SCW (Cuba)	N	(−16.78 ± 0.08)
20	SCW (Cuba)	N	(−16.72 ± 0.06)
21	SCW (Cuba)	N	(−16.93 ± 0.47)
22	SCW (Cuba)	N	(−17.34 ± 0.27)
23	SCW (Cuba)	N	(−16.86 ± 0.04)
24	SCW (Cuba)	N	(−16.89 ± 0.20)
25	SCW (India)	N	(−36.42 ± 0.01)
26	SCW (India)	N	(−36.59 ± 0.03)
27	SCW (India)	N	(−36.88 ± 0.03)
28	SCW (India)	N	(−36.70 ± 0.06)
29	SCW (India)	N	(−36.47 ± 0.17)
30	SCW (India)	N	(−36.78 ± 0.26)
31	SCW (India)	N	(−36.55 ± 0.30)
32	SCW (India)	N	(−36.39 ± 0.06)
33	Batch 1	S	(−30.21 ± 0.19)
34	Batch 1	S	(−29.90 ± 0.15)
35	Batch 1	S	(−29.52 ± 0.17)
36	Batch 1	S	(−29.88 ± 0.17)
37	Batch 1	S	(−29.44 ± 0.04)
38	Batch 1	S	(−29.51 ± 0.22)
39	Batch 2	S	(−33.12 ± 0.52)
40	Batch 2	S	(−32.99 ± 0.45)
41	Batch 2	S	(−32.48 ± 0.53)
42	Batch 2	S	(−32.16 ± 0.55)
43	Batch 2	S	(−32.35 ± 0.28)
44	Batch 2	S	(−32.23 ± 0.32)
45	Batch 2	S	(−32.18 ± 0.32)
46	Batch 2	S	(−32.45 ± 0.20)

Note: [a]Industrial (I), natural (N) or synthetic (S) origin.

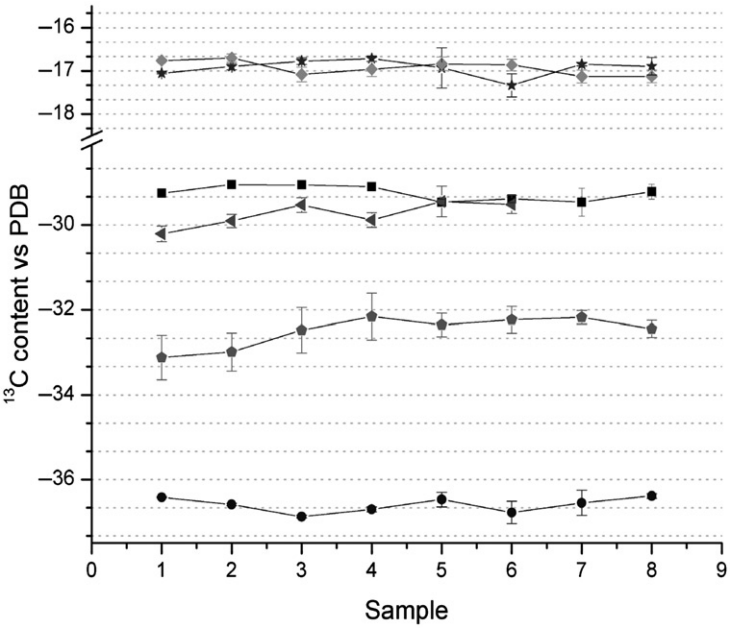


Figure 1. $\delta^{13}C$ for samples vs. PDB plotted with error bars: Sigma–Aldrich (■), Cuba (★), India (●), Giellepi (◆), Batch 1 (◄), Batch 2 (⬟).

Owing to the high commercial value of 1-octacosanol and its unique biological properties, an easy access to this fatty alcohol could stimulate further investigations for its definitive acceptance as food supplement or drug. Moreover, our GC-C-IRMS method enables the discrimination of octacosanol samples origin from different manufacturers and geographical areas.

3. Experimental

3.1. *Materials and methods*

All solvents used were of analytical reagent grade and were purified according to the published procedures (Amarengo & Perrin, 1998). Starting materials, reagents and standards (octadecane) were purchased from Sigma–Aldrich, Fluka and Alpha-Aesar.

For simultaneous MW/US irradiation, the professional oven (Microsynth Milestone – Sorisole, BG) was equipped with a US probe featuring a pyrex horn (frequency 21.4 kHz) (Danacamerini – Torino).

IR spectra were recorded with a Shimadzu FT-IR 8001 spectrophotometer. NMR spectra were recorded with two Bruker Avance spectrometers: 300 MHz and 600 MHz at 300 K; chemical shifts were calibrated to the residual proton resonances of the solvent: $CDCl_3$ ($\delta_H = 7.26$).

Low-resolution mass spectra were recorded on a Finnigan-MAT TSQ70 in electron impact (EI) and chemical ionisation (CI) with isobutane as reactant gas; ESI-mass spectra were recorded on a Waters Micromass ZQ equipped with ESI source.

3.2. *GC-C-IRMS analysis*

Until now, GC has been commonly used to analyse policosanols and other fatty alcohol mixtures with and without derivatisation. Most of the derivatisation methods employed *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA) as silylation reagent. Unfortunately, for the purpose of this work, this procedure adds a relatively high number of carbon atoms to the molecule, which is a complication for IRMS analysis. Recently, some researchers developed GC methods for policosanol analysis that did not involve any derivatisation (Wang et al., 2007). Overall, carbon isotopic ratios were measured with a Trace GC Ultra (Thermo, Italy) equipped with a 95% polydimethylsiloxane capillary column (50 m × 0.2 mm i.d., 0.33 μm coating thickness). The results were expressed in δ^{13}C ‰, corresponding to:

$$1000 \times \frac{^{13}C/^{12}C_{sample} - ^{13}C/^{12}C_{std}}{^{13}C/^{12}C_{std}},$$

where the subscript suffix std refers to the international carbonate standard V-PDB (Coplen, 1994, 1995).

GC-C-IRMS analyses were performed as follows. Octacosanol solutions used for injection were prepared by dissolving 10 mg of product in chloroform (10 mL) in the presence of octadecane (10 mg) as internal standard. Samples were injected at 250°C with a split ratio of 1 : 10. After 2 min, at 150°C (initial temperature of the oven) the temperature was ramped at 25°C min^{-1} up to 300°C and maintained for 6 min in an isotherm. Helium was used as the carrier gas and CO_2 as the reference. The effluent from the column was combusted at 940°C in an alumina tube (0.55 mm inner diameter, 1.55 mm outer diameter, 320 mm long), which acts as an oxidation reactor. The tube contains three wires made of CuO, NiO and Pt, respectively (all 240 mm long and 0.125 mm thick). Nitrogen oxides were reduced to N_2 at 640°C in a reduction furnace made by three twisted copper wires of 0.125 mm diameter. A hygroscopic Naphion® membrane, situated just before the spectrometer, eliminated excess water, avoiding CO_2 protonation and the consequent formation of HCO_2^+ ($m/z = 45$).

Each sample was analysed three times and the results were averaged. The CO_2 reference was calibrated using alkane mixtures supplied by Arndt Schimmelmann of Indiana University with certified δ^{13}C values against V-PDB. The δ^{13}C of 15 linear alkanes, ranging from C_{16} to C_{30}, were calculated by Schimmelmann's method according to Coplen et al.'s (2006) method. Figure 2 reports the GC-C-IRMS spectra of all 1-octacosanol samples.

3.3. *Samples*

As many as six different sets of samples of octacosanol (70–98%) of various origins were investigated. Two industrial sets were supplied by Sigma–Aldrich and by Giellepi Chemicals; two sets were natural wax, extracted from the sugar cane produced in different geographical areas (Cuba, India) and two sets were obtained by synthesis from two different batches. With the aim of checking the repeatability of the synthetic protocol, the synthesis was performed in two different laboratories (Batch 1 and 2), beginning from the starting material of different suppliers.

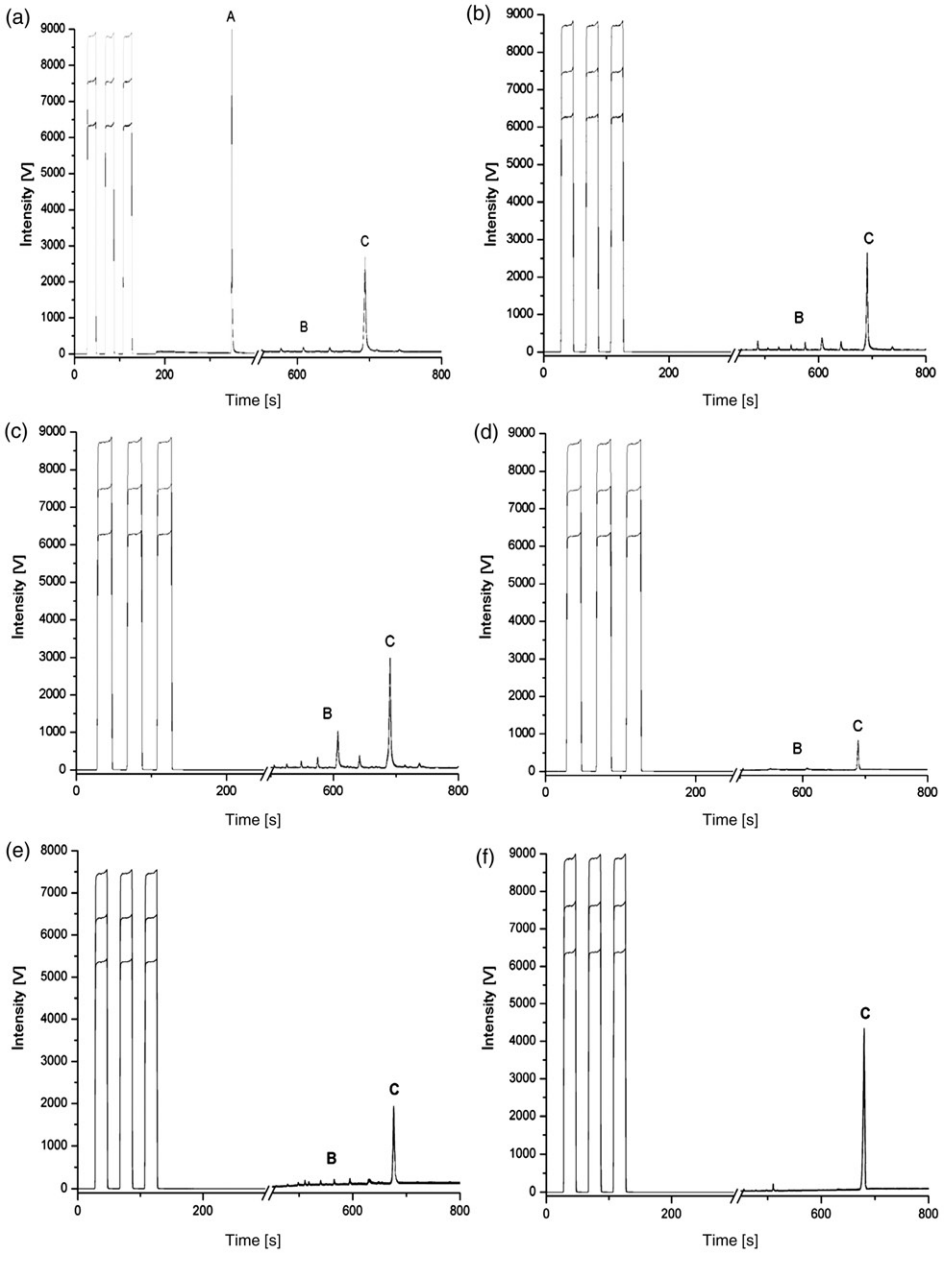


Figure 2. GC-C-IRMS spectra of 1-octacosanol samples from Sigma–Aldrich (a), octacosanol from Cuba (b), octacosanol from India (c), octacosanol from Giellepi (d), octacosanol from synthesis Batch 1 (e) and Batch 2 (f). Peak A represents the internal standard (octadecane), peak B indicates the polialcohols present in smaller percentages than octacosanol and peak C represents octacosanol.

3.4. *Synthetic and purification procedures*

The synthetic protocol is depicted in Scheme 1; some steps have been dramatically improved by means of non-conventional techniques, such as power ultrasound and microwaves alone or combined. Crude products were usually purified by flash chromatography (direct phase) using the *CombiFlash Rf* system (Teledyne-Isco) with hexane/ethyl acetate mixtures as eluent.

3.4.1. *Synthesis of 9-(oxiran-2-yl)nonanoic acid (**1**)*

In a 100 mL round-bottomed flask placed in an ice bath, undecenoic acid (1.8 g, 9.8 mmol) and dichloromethane (30 mL) were added. To the stirred solution (0°C), *m*-chloroperbenzoic acid (MCPBA, 2.7 g, 15.68 mmol) was added portionwise. The reaction mixture was stirred for 4 h at room temperature and monitored by TLC (hexane : ethyl acetate 7 : 3 + 0.5% trifluoroacetic acid as eluent). The mixture was then diluted with dichloromethane (10 mL), washed with water (15 mL, three times) and brine (40 mL), then dried over sodium sulphate. The crude product was purified by flash chromatography using a hexane : ethyl acetate (9 : 1) mixture as eluent. Compound **1** was obtained as a colourless oil (1.9 g, 97% yield). FT-IR (KBr): ν (cm^{-1}) 3502, 2914, 1705, 1469, 1433, 1323, 912, 844, 721; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 2.92 (1H, m, H-10), 2.65 (1H, m, H_A-11), 2.48 (1H, m, H_B-11), 2.37 (2H, t, $J = 7.5$ Hz, H-2), 1.65 (2H, m, H-3), 1.57 (2H, m, H-9), 1.42 (10H, m, H-4, 5, 6, 7, 8). CIMS 201 $[M + 1]^+$ $[C_{11}H_{20}O_3 + H]$.

3.4.2. *Synthesis of 10,11-dihydroxyundecanoic acid (**2**)*

In a 250 mL two-necked conic pyrex flask equipped with an optic-fibre thermometer, compound **1** (1.9 g, 9.5 mmol) and 0.2 N NaOH (90 mL) were added, and simultaneously irradiated with ultrasound and microwaves (US/MW) (Cravotto & Cintas, 2007; Palmisano et al., 2007) for 15 min at 55°C (power: 20 and 50 W respectively). Reaction outcome was checked by TLC with hexane : ethyl acetate 7 : 3 + 0.5% trifluoroacetic acid as eluent. The reacted mixture was cooled to room temperature and brought to pH 5 with 2 N HCl and a whitish amorphous solid was precipitated and collected on a paper filter, then the filtrate was extracted with dichloromethane to recover the residual product. 1.64 g of pure **2** were obtained (79% yield). FT-IR (KBr): ν (cm^{-1}) 3584, 2926, 2361, 2341, 1738, 1713, 1265, 1076, 723. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 4.36 (2H, br, OH), 3.70 (1H, m, H-10), 3.63 (1H, m, H_A-11), 3.49 (1H, m, H_B-11), 2.36 (2H, t, $J = 7.2$ Hz, H-2), 1.66 (2H, m, H-9), 1.53 (2H, m, H-3), 1.45 (2H, m, H-8), 1.27 (8H, m, H-4, 5, 6, 7). CIMS 219 $[M + 1]^+$ $[C_{11}H_{22}O_4 + H]$.

3.4.3. *Synthesis of 10-oxodecanoic acid (**3**)*

To a solution of **2** (1.5 g, 6.9 mmol) in dioxane : water 3 : 1 (60 mL), sodium periodate (3 g, 13.8 mmol) was added and the mixture was sonicated in a cavitating tube (19.2 kHz, 80 W) (Cravotto et al., 2008) at room temperature for 80 min. The reaction was monitored by TLC with hexane : ethyl acetate 7 : 3 + 0.5% trifluoroacetic acid as eluent. The residue was filtered off on a Buckner flask with a sintered glass. The filtrate was concentrated at 50% of the volume under vacuum, poured

into a separatory funnel and extracted with dichloromethane (3 × 20 mL), washed with water (2 × 60 mL) and dried over sodium sulphate. The solvent was removed under vacuum and the crude product was purified by flash chromatography using hexane : ethyl acetate 9 : 1 as eluent, affording **3** as a yellowish solid (1.08 g, 84% yield). FT-IR (KBr): ν (cm^{-1}) 3321, 2928, 2675, 1716, 1464, 1242, 1047, 943, 725. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 9.74 (1H, t, $J = 1.8$ HZ, –CHO), 2.40 (2H, td, $J_t = 8.1$ HZ, $J_d = 1.8$ HZ, H-9), 2.31 (2H, t, $J = 7.5$ HZ, H-2), 1.56 (2H, m, H-8), 1.42 (2H, m, H-7), 1.29 (8H, m, H-3, 4, 5, 6). CIMS 187 $[M + 1]^+$ $[C_{10}H_{18}O_3 + H]$.

3.4.4. *Synthesis of methyl 10-oxodecanoate (**4**)*

To a solution of **3** (1.08 g, 5.8 mmol, 1 eq) in methanol (15 mL), trimethylorthoformate (0.6 g, 5.7 mmol, 1 eq) and methansulfonic acid (10 mg) were added and the solution was irradiated 15 min with MW (50°C, 30 W). The reaction was checked by TLC with hexane : ethyl acetate 7 : 3 + 0.5% trifluoroacetic acid as eluent. The solvent was removed under vacuum, the product was dissolved in dichloromethane (40 mL) and the organic phase was washed with 5% HCl (2 × 30 mL), 5% sodium bicarbonate and brine. The methyl ester containing traces of methyl acetal (less than 2%) was purified by flash chromatography using hexane : ethyl acetate 19 : 1 as eluent, which afforded **4** as a yellow oil: (1.03 g, yield 89%); FT-IR (KBr): ν (cm^{-1}) 2928, 2675, 1745, 1464, 1242, 1047, 943, 725. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 9.74 (1H, t, $J = 1.8$ Hz, –CHO), 3.60 (3H, s, $–OCH_3$), 2.40 (2H, td, $J_t = 8.1$ Hz, $J_d = 1.8$ Hz, H-9), 2.31 (2H, t, $J = 7.5$ Hz, H-2), 1.56 (2H, m, H-8), 1.42 (2H, m, H-7), 1.29 (8H, m, H-3, 4, 5, 6). CIMS 201 $[M + 1]^+$ $[C_{11}H_{20}O_3 + H]$.

3.4.5. *Synthesis of octadecyltriphenylphosphonium bromide (**5**)*

To a 100 mL round-bottomed flask containing stearyl bromide (1 g, 2.9 mmol, 1 eq) dissolved in 25 mL of anhydrous toluene, triphenylphosphine (0.78 g, 2.9 mmol, 1 eq) was added. The solution was heated under reflux overnight in N_2 atmosphere. After cooling at 0°C and adding ethyl ether (30 mL), the phosphonium salt was precipitated and it was collected on a paper filter. Compound **5** was obtained as a whitish product (1.4 g, 81% yield), which was immediately used for the Wittig reaction.

^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.85 (15H, m, –ArH), 1.65 (4H, m, H-1, 2), 1.24 (30H, m, H-3-17), 0.87 (3H, t, $J = 6.4$ Hz, H-18).

3.4.6. *Synthesis of (E,Z)-methyl octacos-10-enoate (**7**)*

Phosphonium salt **5** (1.19 g, 2.0 mmol) was dissolved under N_2 atmosphere in THF (20 mL) and the mixture was cooled to 0°C. Butyllithium (1.6 M, 1.2 eq) was added and the red solution was stirred at room temperature for 15 min. The mixture of the phosphorus ylide (**6**) was again cooled to 0°C and a solution in THF (5 mL) of the aldehyde **4** (0.4 g, 2.0 mmol) was added dropwise and stirred for 2 h at room temperature. The reaction was checked by TLC using hexane:ethyl acetate 7 : 3 as eluent. The reacted mixture was cooled in ice bath and a mixture of diethyl ether (20 mL) and 5% HCl (10 mL) was added. The organic layer was washed with 5% HCl (20 mL), water (30 mL), 5% sodium bicarbonate and brine, then dried over

sodium sulphate, filtered on a paper filter and evaporated under vacuum. The yellow-orange oil was purified by flash chromatography using hexane : ethyl acetate 19 : 1 as eluent, affording compound **7** in good yield as a yellow oil (0.72 g, 82%). FT-IR (KBr): ν (cm^{-1}) = 2926, 1740, 1466, 1180, 1037, 742, 721, 669. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.37 (2H, m, H-10, 11), 3.50 (3H, s, –$COOCH_3$), 2.50 (2H, m, H-2), 2.01 (4H, m, H-9, 12), 1.30 (42H, m, H-3-8, 13-27), 0.89 (3H, t, J = 6.4 Hz, H-28). CIMS 437 $[M + 1]^+$ $[C_{29}H_{56}O_2 + H]$.

3.4.7. *Synthesis of (E,Z)-octacos-10-en-1-ol (**8**)*

In a 50 mL two-necks round-bottomed flask, equipped with a nitrogen inlet, $LiAlH_4$ (164 mg, 4.3 mmol, 3 eq) was dissolved in THF (8 mL) and was cooled to 0°C. A solution of **7** (636 mg, 1.44 mmol) in THF (10 mL) was added dropwise. After the addition was completed, the mixture was kept at room temperature and stirred for 2 h and monitored by TLC using hexane : ethyl acetate 7 : 3 as eluent. After the reaction was completed, 5% HCl (30 mL) was added and extracted with diethyl ether (2 × 20 mL). The organic phase was extracted twice with brine, dried over sodium sulphate, filtered with a paper filter and evaporated under vacuum. The crude product was purified by flash chromatography using hexane : ethyl acetate 19 : 1 mixture as eluent. Compound **8** was obtained as a white solid (529 mg, 90% yield). FT-IR (KBr): ν (cm^{-1}) = 3500, 2926, 1471, 1292, 964, 891, 729, 648. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.37 (2H, m, H-10, 11), 3.63 (2H, t, J = 6.6 Hz, H-1), 2.01 (4H, m, H-9, 12), 1.57 (2H, m, H-2), 1.47 (1H, br, OH), 1.28 (42H, m, H-3-8, 13-27), 0.89 (3H, t, J = 6.3 Hz, H-28). CIMS 409 $[M + 1]^+$ $[C_{28}H_{56}O + H]$.

3.4.8. *Synthesis of octacosan-1-ol (**9**)*

To a solution of octacos-11-en-1-ol (400 mg, 0.98 mmol) in THF (40 mL), 10% of Pd on charcoal (50 mg) was added and poured into a Parr reactor. The reduction was carried out at 80°C and 35 bar H_2 under stirring for 4 h. Similar results were achieved when the reaction was performed in a special US-reactor (21.2 kHz) that works under pressure (Danacamerini – Torino); the reaction was carried out at 40°C and 6 bar H_2 for 3 h. In both cases, after filtration on a celite pad and evaporation under vacuum, a crude product was purified by flash chromatography using hexane : ethyl acetate 19 : 1 as eluent. Compound **9** was obtained as a white powder (358 mg, 89% yield). FT-IR (KBr): ν (cm^{-1}) = 3500, 2923, 1122, 889, 731, 719, 669. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 3.54 (2H, t, J = 6.6 Hz, H-1), 1.57 (2H, m, H-2) 1.46 (5H, m, H-3, 4, OH), 1.29 (46H, m, H-5-27), 0.88 (3H, t, J = 6.3 Hz, H-28). CIMS 411 $[M + 1]^+$ $[C_{28}H_{58}O + H]$.

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