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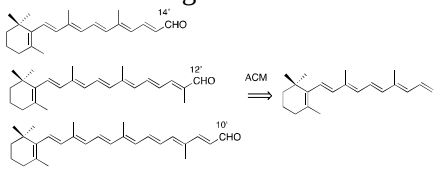

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ABSTRACT

A new synthesis of three apocarotenoids, namely 14'-apo-β-carotenal, 12'-apo-β-carotenal and 10'-apo-β-carotenal, has been achieved that is based on the acyclic cross-metathesis of the hexaene derived from retinal and the corresponding partners. These compounds can be enzymatically converted to their carboxylic acids by the human aldehyde dehydrogenases involved in retinaldehyde oxidation. Their kinetic parameters suggest that these enzymes might play a role in the physiological metabolism of apocarotenoids.

1. Introduction

Carotenoids are a family of natural compounds synthesized by plants, microorganisms and some animals but not by humans.[1] Carotenoids are partly responsible for the colour in nature and play a key role both in the photosynthesis process and the photoprotection of the producing organisms. They can be broadly divided into two classes of chemical compounds: carotenes (e.g., β,β-carotene and lycopene) and their oxygenated derivatives termed xanthophylls (e.g., lutein, zeaxanthin and cryptoxanthin). Both carotenes and xanthophylls exhibit relevant physiological functions, serving as antioxidants in lipophilic environments,[2] a property that might contribute to the prevention of certain human diseases such as cardiovascular, ocular diseases and cancer.[3, 4]

Within carotenoids, the term apocarotenoid is used to designate those with a backbone of less than 40 carbon atoms.[5] Apocarotenoids are formed by the oxidative degradation of one or both termini of carotenoids, a process that is catalyzed by carotenoid cleavage enzymes. The oxidation products derived from dietary β,β-carotene **1** (Figure 1) can be C20 all-*trans*-retinal **2** resulting from the central C15-C15' cleavage catalyzed by BCO1[6, 7] or non-symmetrical β-apocarotenoids obtained by eccentric cleavage catalyzed by BCO2.[8, 9] Carotenoid metabolism appears to be cell compartmentalized,[10] as BCO1 is a cytosolic enzyme,[11] while BCO2 has been associated with mitochondria.[9] BCO2-mediated cleavage is the preferred pathway for xanthophylls.[9]

The metabolism of β,β-carotene **1** to all-*trans*-retinal **2** (Figure 1) promoted by BCO1 is the first step in the production of the natural retinoids (including, but not restricted to, vitamin A, 11-*cis*-retinal and all-*trans*-retinoic acid **3**) required for eliciting the various functions of these compounds in the human body. Thus, enzymes responsible for isomerization and changes in the oxidation state provide the cognate ligands for receptors implicated in vision, cell proliferation, cell differentiation, immunity and development.[4] In particular, the oxidation of all-*trans*-retinal **2** promoted by aldehyde dehydrogenases (ALDHs)[12] generate all-*trans*-retinoic acid **3**, the natural hormone that binds to and activates the retinoic acid receptors (RARs),[13] members of the nuclear receptor superfamily of ligand-inducible transcription factors.[14]

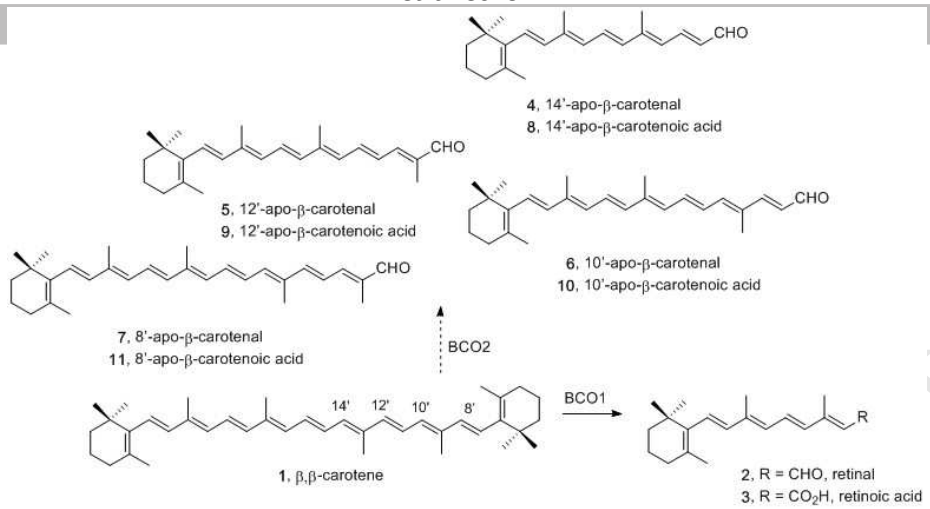


Figure 1. β-apocarotenals **2,4-7** potentially formed by (enzyme-mediated) oxidative cleavage of β,β-carotene **1**, and the corresponding carboxylic acids **3**, **8-11**. The shorter fragments of putative BCO2 cleavage are not shown.

Apocarotenoids (such as **4-11**, Figure 1) have been detected in food and in the blood of animals,[15] and the concentration of some of them are similar to that of all-*trans*-retinoic acid **3**. Their biological functions, however, remain unclear.[16, 17] Recently, it has been proposed that the activity of BCO2-derived metabolites can protect against the damage induced by β,β-carotene **1**. This compound is deemed partially responsible for oxidative stress in the mitochondria, a process that can trigger signaling pathways related to cell survival and proliferation.[17] Moreover, some apocarotenoids, namely 10'-apo-β-carotenoic acid **10**, 12'-apo-β-carotenoic acid **9**,[17] 14'-apo-β-carotenoic acid **8**, 14'-apo-β-carotenal **4**, and 13'-apo-β-carotenone (not shown), have been described as low-affinity agonists potentially acting as endogenous antagonists of RARs,[16, 17] and also were found to regulate other functions, such as the placental lipoprotein biosynthesis.[18]

It is believed that β-apocarotenals can be a potential source of β-apocarotenoic acids, which can be considered as vinylogues of all-*trans*-retinoic acid **3**, through the oxidation of the functional group. However the enzymes involved in these transformations have not been described. Logical candidates are aldehyde dehydrogenases (ALDHs),[12] enzymes that transform aldehydes into carboxylic acids. Within the ALDH superfamily, ALDH1A1, ALDH1A2 and ALDH1A3 are closely related enzymatic forms that catalyze the oxidation of retinaldehydes.[19] In order to shed some light on the metabolism and biological functions of β-apocarotenoids, here we present an efficient synthetic strategy for the preparation of these molecules and their biological characterization as substrates for human ALDHs.

Carotenoids have been traditionally synthesized following two different strategies.[4, 20] The first comprises carbonyl condensation reactions with heteroatom-stabilized carbanions, such as Wittig, Horner–Wadsworth–Emmons and Julia reactions, which form C_{sp2}=C_{sp2} bonds.[21] The second is based on the formation of C_{sp2}–C_{sp2} bonds by palladium-catalyzed cross-coupling reactions[22] (primarily Negishi, Stille, and Suzuki reactions).[23] In both cases, appropriate functionalization of the intermediates for the key reaction is required. Recently, the olefin metathesis reaction[24, 25, 26] has been established as one of the most general and widely applicable synthetic methods for C_{sp2}=C_{sp2} bond formation. Despite the numerous applications of olefin metathesis reactions in the synthesis of natural products,[24] its use in the preparation of conjugated polyene chains has been somehow limited because of concerns about the control of site-selectivity, stereoselectivity, and the stability of polyenes to the reaction conditions. The first application on the synthesis of retinoids[27] and apocarotenoids starting from carotenoids was described by Wojtkielewicz and coworkers, although the products were obtained in very low yields.[28, 29] We[30, 31] and others[32] have also contributed to this field with the synthesis of symmetrical and non-symmetrical carotenoids by dimerization and cross-metathesis processes.[33]

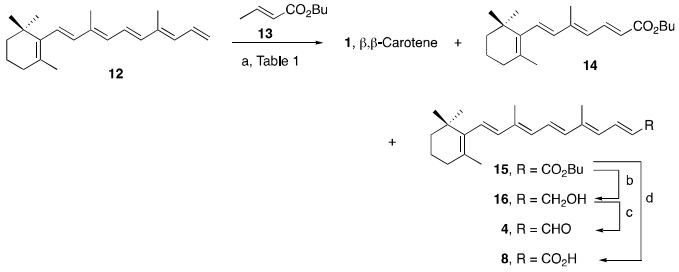
We considered that β-apocarotenoids could also be prepared by cross metathesis of appropriate precursors.[33] We envisioned the synthesis of β-apocarotenoids with different lengths of the polyene chain by acyclic cross-metathesis[33] starting from a common precursor, the already known hexaene **12**[30] and the complementary component functionalized as ester.[30] More specifically, we undertook the synthesis of the three β-apocarotenoids (10'-apo-β-carotenal **4**, 12'-apo-β-carotenal **5** and 14'-apo-β-carotenal **6**; 8'-apo-β-carotenal **7** is a commercial compound) that could potentially by formed by eccentric cleavage of β,β-carotene **1** at the C9'=C10', C11'=C12' and C13'=C14' double bonds.[4] Only the products of cleavage at the C9'=C10' bond have been characterized in mice.[7] However, since β-apocarotenoids can potentially be formed from β,β-carotene **1** by non-enzymatic autoxidation processes, their availability by synthesis can provide useful tools for biochemical research. A general method for oxidation of β,β-carotene **1** with a mixture of $KMnO_4$ and H_2O_2, that provided a mixture of three apo-carotenoids (8'-, 10'- and 12'-apo), was reported half a century ago.[34]

2. Results and discussion

*2.1. Synthesis of 14'-apo-β-carotenal **4***

The synthesis of 14'-apo-β-carotenal **4** started with the cross metathesis[33] of previously described hexaene **12**[30] and commercial butyl (*E*)-but-2-enoate **13**. Four different ruthenium catalysts were tested with toluene as solvent based on the results described by Wojtkielewicz and coworkers[29] and special attention was paid to the reaction time in order to avoid degradation of the formed polyenes (Scheme 1). The use of Neolyst® as catalyst[35] was discouraging as the starting material was fully recovered after 48 h (entry 1, Table 1). The use of Nitro-Grela catalyst[36] allowed to obtain, after 7.5 h, the desired product in 29% yield together with 15% of β,β-carotene **1**, resulting from the competing dimerization of **12**[30] (entry 2, Table 1).

The 2nd generation Grubbs catalyst[26, 37] proved more effective in the metathesis reaction (39% yield for **15**), although β,β-carotene **1** was also obtained as secondary product (entry 3, Table 1). Finally, the use of 2nd generation Hoveyda-Grubbs (HG) catalyst[38] afforded the best results, with a 62% yield of desired **15** and 16% of homodimer **1** obtained after 4.5 h (entry 4, Table 1). The increase of the reaction time turned out to be detrimental since it favored the cross metathesis corresponding formally to the C11=C12 bond, with full consumption of the initially formed apo-β-carotenoid **15** (entry 5, Table 1). Compound **15** was readily transformed into the desired 14'-apo-β-carotenal **4** by Dibal-H reduction followed by oxidation of **16** with MnO_2 under basic conditions (Scheme 1). In addition, saponification of ester **15** with KOH/MeOH at 70 ºC afforded 14'-apo-β-carotenoic acid **8** in 62% yield.



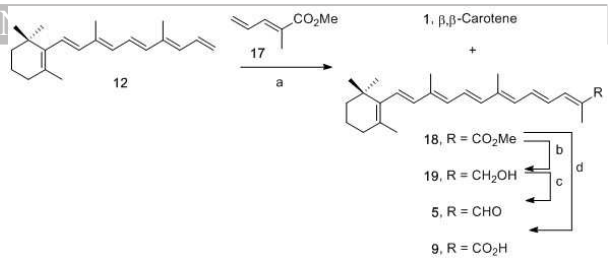
Scheme 1. Reagents and conditions. a) Table 1. b) Dibal-H, THF, -78 ºC, 5h, 71%. c) MnO_2, Na_2CO_3, CH_2Cl_2, 25 ºC, 5h, 88%. d) KOH, MeOH, 70 ºC, 1h, 62%.

Table 1. Olefin cross-metathesis of hexaene **12** and enoate **13**.

	Reagents and conditions	Yield (%)		
		1	**14**	**15**
1	**13** (6 equiv.), Neolyst® (0.15 equiv.), toluene, 25 °C, 48 h	0	0	0
2	**13** (6 equiv.), Nitro-Grela (0.15 equiv.), toluene, 25 °C, 7.5 h	15	0	29
3	**13** (6 equiv.), 2nd gen. Grubbs, toluene, 25 °C, 4.5 h	15	0	39
4	**13** (6 equiv.), 2nd gen. Hoveyda-Grubbs (0.15 equiv.), toluene, 25 °C, 4.5 h	16	1	62
5	**13** (6 equiv.), 2nd gen. Hoveyda-Grubbs (0.15 equiv.), toluene, 25 °C, 24 h	0	36	0

*2.2. Synthesis of 12'-apo-β-carotenal **5***

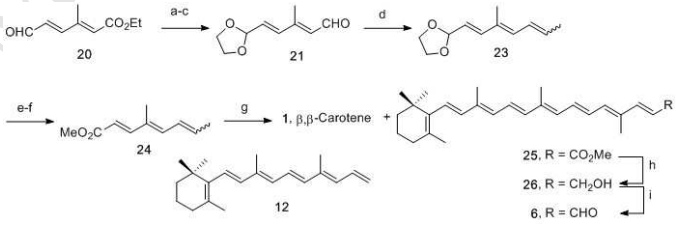
The previously optimized reaction conditions were next applied to the synthesis of 12'-apo-β-carotenal **5**. The reaction of an excess of **17** with hexaene **12** afforded methyl 12'-apo-β-carotenoate **18** in 40% yield along with the product of homodimerization **1** (10%). Some reactant hexaene **12** was also recovered (11%). The geometry of the newly formed double bond was confirmed by NOE experiments. Dibal-H reduction of ester **18** and subsequent oxidation with MnO_2 under basic conditions furnished 12'-apo-β-carotenal **5** (Scheme 2). Ester hydrolysis as described led to 12'-apo-β-carotenoic acid **9** in 53% yield.



Scheme 2. Reagents and conditions. a) 2nd generation HG, toluene, 25 °C, 7h (10% β,β-carotene **1**, 11% starting **12**, 40% methyl 12'-apo-β-carotenoate **18**). b) Dibal-H, THF, -78 °C, 4h, 75%. c) MnO_2, Na_2CO_3, CH_2Cl_2, 25 °C, 4h, 38%. d) KOH, MeOH, 70 ºC, 1h, 53%.

*2.3. Synthesis of 10'-apo-β-carotenal **6***

The anticipated component for the synthesis of 10'-apo-β-carotenal **6**, namely methyl (2*E*,4*E*)-octan-2,4,6-enoate **24**, was prepared by a six step sequence starting from methyl (2*E*,4*E*)-3-methyl-6-oxohexa-2,4-dienoate **20**. Protection of the aldehyde of **20** as dioxolane, followed by the consecutive ester reduction with Dibal-H and MnO_2 oxidation, led to aldehyde **21** in good yields. Wittig olefination with commercially available ethyl triphenylphosphonium iodide **22** provided triene **23** in 80% yield as an inconsequential 1:1 mixture of double bond isomers. Subsequent deprotection of the acetal followed by oxidation of the trienal with MnO_2 and KCN in MeOH provided ester **24** as a mixture of isomers (Scheme 3). An excess of trienyl ester **24** was reacted with hexaene **12** under the conditions already described, which produced the desired methyl 10'-apo-β-carotenoate **25** in 24% yield along with 58% yield of β,β-carotene **1**. A two-step sequence of Dibal-H reduction and MnO_2 oxidation of ester **25** afforded 10'-apo-β-carotenal **6** in a combined 59% yield.



Scheme 3. Reagents and conditions: a) ethylene glycol, *p*TsOH, benzene, 96 ºC, 16h, 99%. b) Dibal-H, THF -78 ºC, 2h, 99%. c) MnO_2, Na_2CO_3, THF, 25 ºC, 5h, 75%. d) ethyl triphenylphosphonium iodide **22**, NaHMDS, THF, -78 ºC, 2.5h, 80%. e) *p*TsOH, acetone, 25 ºC, 17h, 98%. f) MnO_2, KCN, MeOH, 25 ºC, 3.5h, 79%. g) 2nd generation HG, **12**, toluene, 5h (58% β,β-carotene **1**, 49% starting trienoate **24**, 24% methyl 10'-apo-β-carotenoate **25**). h) Dibal-H, THF, -78 ºC. i) MnO_2, Na_2CO_3, CH_2Cl_2, 59% (2 steps).

2.4. ALDH enzymatic assay

The activity of ALDH1A enzymes with apo-β-carotenals **4** and **5** (the study with **6** was not completed due to the low overall yield of the synthetic sequence) was monitored using an improved HPLC-based method for the simultaneous detection of substrates and reaction products. In all cases, concomitant consumption of apo-β-carotenals **4** and **5**, and production of the corresponding apo-β-carotenoic acids **8** and **9**, respectively, were observed (see Figure 2 and Supporting Information). Thus, ALDH1A enzymes turned out to be active with 14'- and 12'-apo-β-carotenals (**4** and **5**).

Table 2. Kinetic constants of human ALDH1A enzymes with apo-β-carotenals.[a]

	ALDH1A1			ALDH1A2			ALDH1A3		
Substrate	K_m (μM)	k_{cat} (min^{-1})	k_{cat}/K_m ($mM^{-1}\cdot min^{-1}$)	K_m (μM)	k_{cat} (min^{-1})	k_{cat}/K_m ($mM^{-1}\cdot min^{-1}$)	K_m (μM)	k_{cat} (min^{-1})	k_{cat}/K_m ($mM^{-1}\cdot min^{-1}$)
12'-apo-β-carotenal **5**	NS	–	44	1.8 ± 0.2	8.5 ± 0.2	4800 ± 600	3.0 ± 0.4	1.3 ± 0.1	450 ± 62
14'-apo-β-carotenal **4**	12 ± 2	3.1 ± 0.2	257 ± 47	5.0 ± 0.5	4.7 ± 0.2	930 ± 93	NS	–	303

[a]Enzymatic activity was measured in 50 mM HEPES, pH 8.0, 0.5 mM NAD^+, including 0.5 mM EDTA and 0.5 mM DTT for ALDH1A1 and ALDH1A2, or 30 mM $MgCl_2$ and 5 mM DTT for ALDH1A3, at 37 °C. Apocarotenoic acid production was quantified by using an HPLC-based method. NS, no saturation (60 μM was the highest substrate concentration tested in the assay), where the k_{cat}/K_m value was calculated from the slope of V/[E] vs [S] plot.

Table 2 compares the kinetic constants of the ALDH1A enzymes with 12'- and 14'-apo-β-carotenals (**5** and **4**), showing some differential substrate specificity. In general, K_m values were in the micromolar range, except for ALDH1A1 and ALDH1A3, which were not saturated with 12'-apo-β-carotenal **5** and 14'-apo-β-carotenal **4**, respectively (Figure 3). While ALDH1A1 showed higher specificity for 14'-apo-β-carotenal **4**, ALDH1A2 exhibited higher catalytic efficiency for 12'-apo-β-carotenal **5**. This catalytic efficiency is about ten-fold higher than those of ALDH1A1 and ALDH1A3, and similar to that observed for these enzymes with all-*trans*-retinaldehyde **2**. The k_{cat} values are in the same range in all the experiments performed, but lower than those for all-*trans*-retinaldehyde **2** (data not shown; to be reported elsewhere).

To date, the enzymatic activity of ALDH with these compounds had only been reported in microorganisms.[39] This is the first report on the apocarotenal dehydrogenase activity of human ALDH1A enzymes.

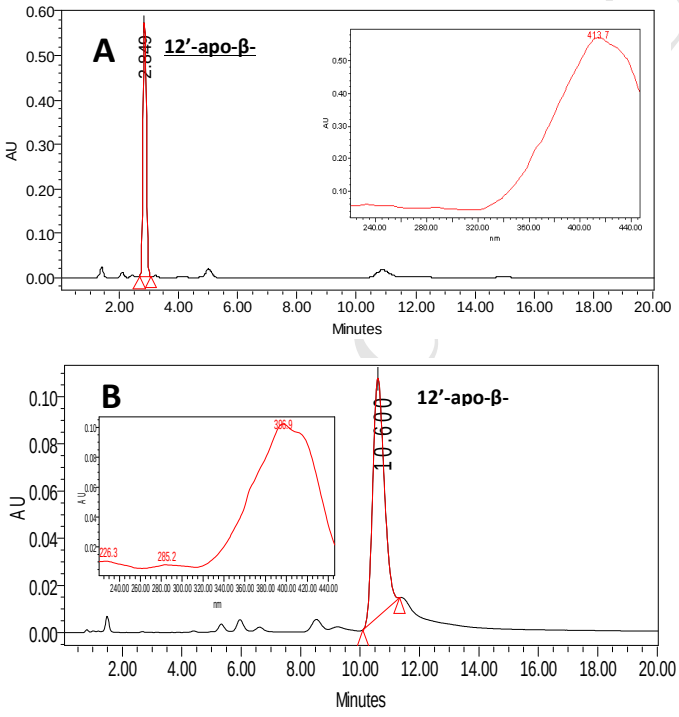


Figure 2. HPLC elution profiles of 12'-apo-β-carotenal 5 (A) and 12'-apo-β-carotenoic acid 9 (B). Stock solutions of apocarotenoids were prepared in ethanol and 10 μM of either compound was injected. Each compound was separated by liquid chromatography on a NovaPak® silica gel column (4 μm, 3.9 x 150 mm) in hexane/*tert*-butyl methyl ether (96:4, v/v) mobile phase, at a flow rate of 2 mL/min using a Waters Alliance 2695 HPLC. Elution of 12'-apo-β-carotenal **5** and 12'-apo-β-carotenoic acid **9** was monitored with a Waters 2996 photodiode array detector at 415 and 400 nm, respectively. The retention times for 12'-apo-β-carotenal **5** and 12'-apo-β-carotenoic acid **9** were 2.85 and 10.60 min, respectively. Insets: UV-vis absorption spectra of the two compounds.

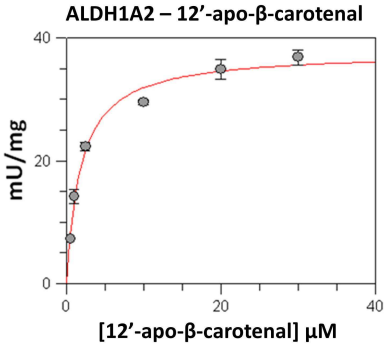


Figure 3. Representative Michaelis-Menten kinetics of ALDH1A2 with 12'-apo-β-carotenal 5. The reaction was carried out in 50 mM HEPES, 0.5 mM EDTA, 0.5 mM DTT, pH 8.0, at 37 °C. The initial rates were measured with at least six different substrate concentrations. Experimental values were adjusted to the Michaelis-Menten equation using the non-linear regression program GraFit 5.0 (Eritacus software).

3. Conclusion

We have developed a new stereoselective synthesis of three apocarotenoids (14'-apo-β-carotenal **4**, 12'-apo-β-carotenal **5** and 10'-apo-β-carotenal **6**) and demonstrated that the olefin metathesis protocol is a valid synthetic method for accessing these conjugated polyenes. Whereas the acyclic cross-metathesis of the common required hexaene was efficiently performed with the shorter enoate and dienoate partners, the chemoselectivity with the longer trienoates is poor due to the presence of several trans-disubstituted olefins that can compete. Previous syntheses of these apo-β-carotenoids have in general featured carbonyl condensation reactions, such as Wittig and aldol condensations (**4**[39], **5**[39, 40] **2**-**4**[17]) or enol ether reactions with acetals followed by elimination and hydrolysis in the case of **4**[41].

We have shown that human retinaldehyde dehydrogenases are capable of using apo-β-carotenals as substrates with good catalytic efficiency. ALDH1A1 and ALDH1A2 exhibit higher substrate specificity for **4** and **5**, respectively, while ALDH1A3 shows similar specificity for the two compounds. Overall, the substrate specificity of ALDH1 enzymes with apo-β-carotenals is comparable to that with all-*trans*-retinaldehyde **2**, indicating that

these enzymes might play a physiological role in the metabolism of apocarotenoids.

4. Experimental Section

General experimental procedures. See E.S.I.

*4.1. Butyl (2E,4E,6E,8E,10E)-5,9-Dimethyl-11-(2,6,6-trimethylcyclohex-1-en-1-yl)undeca-2,4,6,8,10-pentaenoate (butyl 14'-β-apocarotenoate) (**15**).*

General procedure for the olefin metathesis reaction. To a solution of (1*E*,3*E*,5*E*,7*E*)-2-(3,7-dimethyldeca-1,3,5,7,9-pentaenyl)-1,3,3-trimethylcyclohex-1-ene **12** (14 mg, 0.049 mmol) and butyl (*E*)-but-2-enoate **13** (46.4 μL, 0.294 mmol) in toluene (0.25 mL), 2nd generation Hoveyda-Grubbs catalyst (0.46 mg, 0.0073 mmol) was added and the reaction mixture was thoroughly degassed. After stirring at 25 °C for 4.5 h, the mixture was filtered through a Celite™ pad, which was washed with Et_2O and the solvent was evaporated. The residue was purified by column chromatography (silica gel, 99:1 hexane/EtOAc) to afford, in order of elution, 2.2 mg (16%) of a red solid identified as *β,β*-carotene **1**, 11.8 mg (62%) of a red oil identified as butyl (2*E*,4*E*,6*E*,8*E*,10*E*)-5,9-dimethyl-11-(2,6,6-trimethylcyclohex-1-en-1-yl)undeca-2,4,6,8,10-pentaenoate **15** and 0.2 mg (1%) of a yellow oil identified as butyl (2*E*,4*E*,6*E*)-5-methyl-7-(2,6,6-trimethylcyclohex-1-en-1-yl) hepta-2,4,6-trienoate **14**.

Data for *β,β*-carotene (**1**): ^{1}H-NMR (400 MHz, C_6D_6): δ 6.79 (dd, *J* = 14.8, 11.4 Hz, 2H, $2H_{11}$), 6.70-6.67 (m, 2H, H_{15}), 6,49 (d, *J* = 14.8 Hz, 2H, H_{12}), 6.42 - 6.31 (m, 8H, $2H_7$ + $2H_8$+ $2H_{10}$ + $2H_{14}$), 1.99 (t, *J* = 5.6 Hz, 4H, 2 x CH_2), 1.94 (s, 6H, 2 x CH_3), 1.88 (s, 6H, 2 x CH_3), 1.82 (s, 6H, 2 x CH_3), 1.64 - 1.56 (m, 4H, 2 x CH_2), 1.52 - 1.49 (m, 4H, 2 x CH_2), 1.16 (s, 12H, 4 x CH_3) ppm. HRMS (ESI$^+$): Calcd. for $C_{40}H_{56}$ ([M+H]$^+$), 536.4376; found, 536.4376. UV (MeOH): λ_{max} 449, 241 nm.

Data for butyl (2*E*,4*E*,6*E*,8*E*,10*E*)-5,9-dimethyl-11-(2,6,6-trimethylcyclohex-1-en-1-yl)undeca-2,4,6,8,10-pentaenoate (**15**): ^{1}H-NMR (400 MHz, $CO(CD_3)_2$): δ 7.71 (dd, *J* = 15.0, 12.0 Hz, 1H, H_{15}), 7.02 (dd, *J* = 15.0, 11.4 Hz, 1H, H_{11}), 6.48 (d, *J* = 15.0 Hz, 1H, H_{12}), 6.34 (d, *J* = 11.8 Hz, 1H, H_{14}), 6.42 - 6.18 (m, 3H, H_7 + H_8 + H_{10}), 5.97 (d, *J* = 15.0 Hz, 1H, $H_{15'}$), 4.16 (t, *J* = 6.6 Hz, 2H, O-CH_2-), 2.13 (s, 3H, CH_3), 2.10 - 2.06 (m, 2H, CH_2), 2.06 (s, 3H, CH_3), 1.75 (s, 3H, CH_3), 1.72 - 1.61 (m, 4H, 2 x CH_2), 1.54 - 1.48 (m, 2H, CH_2), 1.47 - 1.39 (m, 2H, CH_2), 1.07 (s, 6H, 2 x CH_3), 0.97 (t, *J* = 7.4 Hz, 3H, $CH_2\underline{CH_3}$) ppm. ^{13}C-NMR (100 MHz, $(CD_3)_2CO$): 167.4 (s), 145.2 (s), 140.8 (d), 138.8 (s), 138.7 (s), 138.6 (d), 137.0 (d), 131.4 (d), 130.1 (s), 129.5 (d), 129.4 (d), 128.4 (d), 121.4 (d), 64.4 (t), 40.4 (t), 34.9 (s), 33.6 (t), 31.6 (t), 29.3 (q, 2x), 21.9 (q), 19.9 (t), 19.8 (t), 14.0 (q), 13.1 (q), 12.9 (q) ppm. HRMS (ESI$^+$): Calcd. for $C_{26}H_{39}O_2$ ([M+H]$^+$), 383.2945; found, 383.2946. IR (NaCl): υ 2957 (m, C-H), 2928 (m, C-H), 2866 (w, C-H), 1708 (s, C=O), 1615 (m), 1563 (m), 1135 (s), 970 (m) cm^{-1}. UV (MeOH): λ_{max} 394 nm.

Data for butyl (2*E*,4*E*,6*E*)-5-methyl-7-(2,6,6-trimethylcyclohex-1-en-1-yl)hepta-2,4,6-trienoate (**14**): ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.71 (dd, *J* = 15.0, 11.8 Hz, 1H, H_3), 6.38 (d, *J* = 16.1 Hz, 1H, H_6 or H_7), 6.18 - 6.10 (m, 2H, H_6 or H_7 + H_4), 5.87 (d, *J* = 15.5 Hz, 1H, H_2), 4.16 (t, *J* = 6.7 Hz, 2H, O-CH_2), 2.04 (s, 3H, CH_3), 2.05 - 1.95 (m, 2H, CH_2), 1.71 (s, 3H, CH_3), 1.68 - 1.58 (m, 4H, 2 x CH_2), 1.50 - 1.37 (m, 4H, 2 x CH_2), 1.03 (s, 6H, 2 x CH_3), 0.95 (t, *J* = 7.4 Hz, 3H, $CH_2\underline{CH_3}$) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$): δ 167.8 (s), 144.4 (s), 140.7 (d), 137.6 (s), 136.9 (d), 130.9 (s), 130.8 (d), 127.4 (d), 120.2 (d), 64.3 (t), 39.7 (t), 34.4 (s), 33.3 (t), 30.9 (t), 29.1 (q, 2x), 21.9 (q), 19.4 (t), 19.3 (t), 13.9 (q), 13.2 (q) ppm. HRMS (ESI$^+$): Calcd. for $C_{21}H_{33}O_2$ ([M+H]$^+$), 317.2475; found, 317.2472. IR (NaCl): υ 2958 (m, C-H), 2929 (m, C-H), 1711 (s, C=O), 1598 (m), 1144 (m), 977 (m) cm^{-1}. UV (MeOH): λ_{max} 329 nm.

*4.2. (2E,4E,6E,8E,10E)-5,9-Dimethyl-11-(2,6,6-trimethylcyclohex-1-en-1-yl) undeca-2,4,6,8,10-pentaenal (14'-apo-β-carotenal) (**4**).*

General procedure for Dibal-H reduction of esters. To a cooled (-78 °C) solution of (2*E*,4*E*,6*E*,8*E*,10*E*)-5,9-dimethyl-11-(2,6,6-trimethylcyclohex-1-en-1-yl)undeca-2,4,6,8,10-pentaenoate **15** (13.2 mg, 34.5 mmol) in THF (0.175 mL) was added Dibal-H (0.103 mL, 1M in hexanes, 0.103 mmol). After stirring for 5 h at -78 °C, H_2O was added and the mixture was extracted with EtOAc (3x). The combined organic layers were washed with brine (3x), dried (Na_2SO_4) and the solvent was evaporated. The residue was purified by column chromatography (silica gel, 93:4:3 hexane/EtOAc/Et_3N) to afford 7.7 mg (71%) of a yellow oil identified as (2*E*,4*E*,6*E*,8*E*,10*E*)-5,9-dimethyl-11-(2,6,6-trimethylcyclohex-1-en-1-yl)undeca-2,4,6,8,10-pentaene-1-ol **16**, which was used without further purification.

General procedure for MnO_2 oxidation of alcohols. To a cooled (0 ºC) solution of (2*E*,4*E*,6*E*,8*E*,10*E*)-5,9-dimethyl-11-(2,6,6-trimethylcyclohex-1-en-1-yl)undeca-2,4,6,8,10-pentaene-1-ol **16** (28.5 mg, 0.0912 mmol) in CH_2Cl_2 (1.7 mL) MnO_2 (0.143 g, 1.64 mmol) and Na_2CO_3 (0.174 g, 1.64 mmol) were added. After stirring for 5h at 25 ºC, the reaction mixture was filtered through a Celite™ pad and the solvent was removed. The residue was purified by column chromatography (silica gel, 95:2:3 hexane/EtOAc/Et_3N) to afford 24.8 mg (88%) of a yellow oil identified as (2*E*,4*E*,6*E*,8*E*,10*E*)-5,9-dimethyl-11-(2,6,6-trimethylcyclohex-1-en-1-yl)undeca-2,4,6,8,10-pentaenal **4**. ^{1}H-NMR (400 MHz, $(CD_3)_2CO$): δ 9.65 (d, *J* = 8.0 Hz, 1H, $H_{14'}$), 7.76 (dd, *J* = 14.9, 12.0 Hz, 1H, H_{15}), 7.07 (dd, *J* = 15.0, 11.5 Hz, 1H, H_{11}), 6.50 (d, *J* = 15.0 Hz, 1H, H_{12}), 6.48 (d, *J* = 11.8 Hz, 1H, H_{14}), 6.38 - 6.18 (m, 3H, H_7 + H_8 + H_{10}), 6.16 (dd, *J* = 14.9, 8.0 Hz, 1H, $H_{15'}$), 2.17 (s, 3H, CH_3), 2.08 - 2.04 (m, 2H, CH_2), 2.05 (s, 3H, CH_3), 1.73 (s, 3H, CH_3), 1.68 - 1.60 (m, 2H, CH_2), 1.52 - 1.47 (m, 2H, CH_2), 1.05 (s, 6H, 2 x CH_3) ppm. ^{13}C-NMR (100 MHz, $(CD_3)_2CO$): δ 193.7 (d), 148.4 (d), 147.2 (s), 139.5 (s), 138.5 (s), 138.4 (d), 136.8 (d), 131.9 (d), 131.4 (d), 130.5 (d), 130.2 (s), 129.7 (d), 128.8 (d), 40.3 (t), 34.8 (s), 33.6 (t), 29.3 (q, 2x), 22.0 (q), 19.9 (t), 13.2 (q), 12.9 (q) ppm. HRMS (ESI$^+$): Calcd. for $C_{22}H_{30}O$ ([M+H]$^+$), 311.2369; found, 311.2376. IR (NaCl): υ 2926 (m, C-H), 2863 (w, C-H), 1670 (s, C=O), 1559 (m), 1124 (m), 967 (m) cm^{-1}. UV (MeOH): λ_{max} 402 nm (ε = 45 950 mol^{-1} L cm^{-1}).

*4.3. (2E,4E,6E,8E,10E)-5,9-Dimethyl-11-(2,6,6-trimethylcyclohex-1-en-1-yl)undeca-2,4,6,8,10-pentaenoic Acid (14'-β-apocarotenoic acid) (**8**)*

General procedure for hydrolysis of esters with KOH. To a solution of (2*E*,4*E*,6*E*,8*E*,10*E*)-5,9-dimethyl-11-(2,6,6-trimethylcyclohex-1-en-1-yl)undeca-2,4,6,8,10-pentaenoate **15** (19.6 mg, 51.2 mmol) in MeOH (3.53 mL) was added KOH (0.82 mL, 2M in H_2O, 1.64 mmol). After stirring at 70 °C for 1 h, the reaction mixture was cooled down to 25 °C, CH_2Cl_2 and brine were added and the layers were separated. The organic layer was washed with H_2O (3x). The combined aqueous layers were acidified until acidic pH and extracted with CH_2Cl_2 (3x). The combined organic layers were dried (Na_2SO_4) and the solvent was evaporated. The residue was purified by column chromatography (silica gel, 70:30 hexane/EtOAc) to afford 10.4 mg (62%) of a orange solid identified as (2*E*,4*E*,6*E*,8*E*,10*E*)-5,9-

dimethyl-11-(2,6,6-trimethylcyclohex-1-en-1-yl)undeca-2,4,6,8,10-pentaenoic acid **8**. ^{1}H-NMR (400 MHz, $(CD_3)_2CO$): δ 7.68 (dd, *J* = 14.9, 12.0 Hz, 1H, H_{15}), 6.98 (dd, *J* = 15.0, 11.5 Hz, 1H, H_{11}), 6.45 (d, *J* = 15.0 Hz, 1H, H_{12}), 6.35 (d, *J* = 12.1 Hz, 1H, H_{14}), 6.38 - 6.16 (m, 3H, H_7 + H_8 + H_{10}), 5.92 (d, *J* = 15.0 Hz, 1H, $H_{15'}$), 2.09 (s, 3H, CH_3), 2.07 - 1.97 (m, 2H, CH_2), 2.02 (s, 3H, CH_3), 1.71 (s, 3H, CH_3), 1.68 - 1.57 (m, 2H, CH_2), 1.51 - 1.46 (m, 2H, CH_2), 1.04 (s, 6H, 2 x CH_3) ppm. ^{13}C-NMR (100 MHz, $(CD_3)_2CO$): 168.0 (s), 145.0 (s), 141.2 (d), 138.8 (s), 138.6 (s), 138.5 (d), 137.1 (d), 131.4 (d), 130.1 (s), 129.4 (d), 129.3 (d), 128.4 (d), 121.6 (d), 40.4 (t), 34.9 (s), 33.6 (t), 29.3 (q, 2x), 21.9 (q), 19.9 (t), 13.1 (q), 12.9 (q) ppm. HRMS (ESI^+): Calcd. for $C_{22}H_{31}O_2$ ($[M+H]^+$), 327.2319; found, 327.2316. IR (NaCl): υ 2924 (m, C-H), 2856 (w, C-H), 1680 (s, C=O), 1560 (m), 1162 (s), 969 (m) cm^{-1}. UV (MeOH): λ_{max} 375 (ε = 46 100 mol^{-1} L cm^{-1}).

*4.4. Methyl (2E,4E,6E,8E,10E,12E)-2,7,11-Trimethyl-13-(2,6,6-trimethylcyclohex-1-en-1-yl)trideca-2,4,6,8,10,12-hexaenoate (methyl 12'-apo-β-carotenoate) (**18**).*

Following the general procedure for the olefin metathesis, the reaction of (1,3,3-trimethyl-2-((1*E*,3*E*,5*E*,7*E*)-3,7-dimethyldeca-1,3,5,7,9-pentaenyl)cyclohex-1-ene **12** (21 mg, 0.074 mmol) with methyl (*E*)-2-methylpenta-2,4-dienoate **17** (37.5 mg, 0.298 mmol) and 2nd generation Hoveyda-Grubbs catalyst (7 mg, 0.011 mmol) in toluene (0.8 mL) at 25 °C for 7 h, afforded, after purification by column chromatography (silica gel, gradient from 100:0 to 99:1 hexane/EtOAc), in order of elution, 2.4 mg (11%) of a yellow oil identified as 1,3,3-trimethyl-2-((1*E*,3*E*,5*E*,7*E*)-3,7-dimethyldeca-1,3,5,7,9-pentaenyl)cyclohex-1-ene **12**, 1.9 mg (10%) of a red solid identified as β-β-carotene **1** and 10.3 mg (40%) of a red solid identified as methyl (2*E*,4*E*,6*E*,8*E*,10*E*,12*E*)-2,7,11-trimethyl-13-(2,6,6-trimethylcyclohex-1-en-1-yl)trideca-2,4,6,8,10,12-hexaenoate **18**. ^{1}H-NMR (400 MHz, $(CD_3)_2CO$): δ 7.34 (d, *J* = 11.8 Hz, 1H, $H_{14'}$), 7.10 (dd, *J* = 14.3, 12.0 Hz, 1H, H_{15}), 6.89 (dd, *J* = 14.9, 11.5 Hz, 1H, H_{11}), 6.69 (dd, *J* = 14.2, 12.1 Hz, 1H, $H_{15'}$), 6.46 (d, *J* = 15.0 Hz, 1H, H_{12}), 6.39 (d, *J* = 11.8 Hz, 1H, H_{14}), 6.34 – 6.16 (m, 3H, H_7 + H_8 + H_{10}), 3.74 (s, 3H, OCH_3), 2.09 (s, 3H, CH_3), 2.10 - 2.02 (m, 2H, CH_2), 2.03 (s, 3H, CH_3), 1.99 (s, 3H, CH_3), 1.75 (s, 3H, CH_3), 1.70 - 1.62 (m, 2H, CH_2), 1.54 - 1.48 (m, 2H, CH_2), 1.07 (s, 6H, 2 x CH_3) ppm. ^{13}C-NMR (100 MHz, $(CD_3)_2CO$): δ 168.8 (s), 140.8 (s), 139.4 (d), 138.8 (d), 138.7 (s), 137.8 (d), 137.6 (s), 137.0 (d), 132.3 (d), 131.8 (d), 129.9 (s), 128.9 (d), 127.8 (d), 127.7 (d), 126.9 (s), 51.9 (q), 40.4 (t), 34.9 (s), 33.6 (t), 29.3 (q, 2x), 22.0 (q), 19.9 (t), 13.0 (q), 12.9 (q), 12.8 (q) ppm. HRMS (ESI^+): Calcd. for $C_{26}H_{37}O_2$ ($[M+H]^+$), 381.2788; found, 381.2786. IR (NaCl): υ 2926 (m, C-H), 2862 (w, C-H), 1703 (m, C=O), 1235 (m) cm^{-1}. UV (MeOH): λ_{max} 410 nm.

*4.5. (2E,4E,6E,8E,10E,12E)-2,7,11-trimethyl-13-(2,6,6-trimethylcyclohex-1-en-1-yl)trideca-2,4,6,8,10,12-hexaenal (12'-apo-β-carotenal) (**5**).*

Following the general procedure for Dibal-H reduction of esters, the reaction of methyl (2*E*,4*E*,6*E*,8*E*,10*E*,12*E*)-2,7,11-trimethyl-13-(2,6,6-trimethylcyclohex-1-en-1-yl)trideca-2,4,6,8,10,12-hexaenoate **18** (15.7 mg, 0.041 mmol) with Dibal-H (0.124 mL, 0.124 mmol, 1M in hexanes) in THF (0.209 mL) at -78 ºC for 4h, afforded, after purification by column chromatography (silica gel, 90:7:3 hexane/EtOAc/Et_3N) 10.9 mg (75%) of a reddish oil identified as (2*E*,4*E*,6*E*,8*E*,10*E*,12*E*)-2,7,11-trimethyl-13-(2,6,6-trimethylcyclohex-1-en-1-yl)trideca-2,4,6,8,10,12-hexaene-1-ol **19**, which was used without further purification.

Following the general procedure for MnO_2 oxidation of alcohols, the reaction of (2*E*,4*E*,6*E*,8*E*,10*E*,12*E*)-2,7,11-trimethyl-13-(2,6,6-trimethylcyclohex-1-en-1-yl)trideca-2,4,6,8,10,12-hexaene-1-ol **19** (8.4 mg, 0.0024 mmol) with MnO_2 (37.3 mg, 0.429 mmol) and Na_2CO_3 (45.4 mg, 0.429 mmol) in CH_2Cl_2 (0.443 mL) at 25 ºC for 4h, afforded, after purification by column chromatography (silica gel, 95:2:3 hexane/EtOAc/Et_3N) 3.2 mg (38%) of a orange-reddish oil identified as (2*E*,4*E*,6*E*,8*E*,10*E*,12*E*)-2,7,11-trimethyl-13-(2,6,6-trimethylcyclohex-1-en-1-yl)trideca-2,4,6,8,10,12-hexaenal **5**. ^{1}H-NMR (400 MHz, $(CD_3)_2CO$): δ 9.46 (s, 1H, $H_{12'}$), 7.24 (dd, *J* = 14.1, 12.1 Hz, 1H, H_{15}), 7.13 (d, *J* = 11.8 Hz, 1H, $H_{14'}$), 6.92 (dd, *J* = 14.9, 11.5 Hz, 1H, H_{11}), 6.84 (dd, *J* = 14.1, 11.9 Hz, 1H, $H_{15'}$), 6.46 (d, *J* = 15.2 Hz, 1H, H_{12}), 6.42 (d, *J* = 12.2 Hz, 1H, H_{14}), 6.32 – 6.15 (m, 3H, H_7 + H_8 + H_{10}), 2.08 (s, 3H, CH_3), 2.09 - 2.01 (m, 2H, CH_2), 2.01 (s, 3H, CH_3), 1.83 (s, 3H, CH_3), 1.72 (s, 3H, CH_3), 1.71 - 1.55 (m, 2H, CH_2), 1.56 - 1.44 (m, 2H, CH_2), 1.04 (s, 6H, 2 x CH_3) ppm. ^{13}C-NMR (100 MHz, $(CD_3)_2CO$): δ 194.4 (d), 149.3 (d), 142.3 (s), 138.7 (d), 138.6 (s), 138.5 (d), 138.2 (s), 137.6 (d), 137.5 (s), 132.1 (d), 131.7 (d), 130.0 (s), 128.6 (d), 128.4 (d), 128.1 (d), 40.3 (t), 34.9 (s), 33.6 (t), 29.3 (q, 2x), 22.0 (q), 19.9 (t), 13.0 (q), 12.8 (q), 9.5 (q) ppm. HRMS (ESI^+): Calcd. for $C_{25}H_{35}O$ 351.2682; found, 351.2695. IR (NaCl): υ 2924 (m, C-H), 2861 (w, C-H), 1667 (s, C=O), 1544 (m), 1185 (m) cm^{-1}. UV (MeOH): λ_{max} 424 nm (ε = 69 600 mol^{-1} L cm^{-1}).

*4.6. (2E,4E,6E,8E,10E,12E)-2,7,11-Trimethyl-13-(2,6,6-trimethylcyclohex-1-en-1-yl)trideca-2,4,6,8,10,12-hexaenoic Acid (12'-apo-β-carotenoic acid) (**9**).*

Following the general procedure for hydrolysis of esters, the reaction of (2*E*,4*E*,6*E*,8*E*,10*E*,12*E*)-2,7,11-trimethyl-13-(2,6,6-trimethylcyclohex-1-en-1-yl)trideca-2,4,6,8,10,12-hexaenoate **18** (10.1 mg, 0.026 mmol) with KOH (0.425 mL, 2M in H_2O, 0.85 mmol) in MeOH (1.83 mL) at 70 °C for 1 h, afforded, after purification by column chromatography (silica gel, 70:30 hexane/EtOAc) 5.1 mg (53%) of a red solid identified as (2E,4E,6E,8E,10E,12E)-2,7,11-trimethyl-13-(2,6,6-trimethylcyclohex-1-en-1-yl)trideca-2,4,6,8,10,12-hexaenoic acid **9**. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.42 (d, *J* = 11.8 Hz, 1H, $H_{14'}$), 6.95 (dd, *J* = 14.0, 12.1 Hz, 1H, H_{15}), 6.76 (dd, *J* = 14.9, 11.5 Hz, 1H, H_{11}), 6.54 (dd, *J* = 13.8, 12.1 Hz, 1H, $H_{15'}$), 6.36 (d, *J* = 15.0 Hz, 1H, H_{12}), 6.27 (d, *J* = 12.4 Hz, 1H, H_{14}), 6.23 – 6.10 (m, 3H, H_7 + H_8 + H_{10}), 2.02 (s, 3H, CH_3), 1.99 (s, 3H, CH_3), 2.10 – 1.99 (m, 2H, CH_2), 1.72 (s, 3H, CH_3), 1.66 - 1.57 (m, 2H, CH_2), 1.51 - 1.47 (m, 2H, CH_2), 1.03 (s, 6H, 2 x CH_3) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$): δ 173.1 (s), 141.0 (d), 140.8 (s), 138.0 (s), 137.7 (d), 137.6 (s), 137.1 (d), 136.6 (d), 131.1 (d), 130.6 (d), 129.8 (s), 127.8 (d), 127.7 (d), 127.2 (d), 125.2 (s), 39.8 (t), 34.4 (s), 33.3 (t), 29.2 (q, 2x), 21.9 (q), 19.4 (t), 13.1 (q), 13.0 (q), 12.6 (q) ppm. HRMS (ESI^+): Calcd. for $C_{25}H_{35}O_2$ ($[M+H]^+$), 367.2632; found, 367.2629. IR (NaCl): υ 2924 (m, C-H), 2858 (w, C-H), 1670 (s, C=O), 1419 (m), 1248 (m), 968 (m) cm^{-1}. UV (MeOH): λ_{max} 402 (ε = 61 450 mol^{-1} L cm^{-1}).

*4.7. Ethyl (2E,4E)-5-(1,3-Dioxolan-2-yl)-3methylpenta-2,4-dienoate (**20a**).*

To a 500 mL round-bottomed flask armed with a Dean-Stark trap, were added ethyl (2*E*,4*E*) 3-methyl-6-oxohexa-2,4-dienoate **20** (2.5 g, 14.8 mmol), benzene (256.3 mL), *p*-TsOH (170 mg, 0.89 mmol) and ethyleneglycol (8.7 mL, 156 mmol), and the resulting mixture was heated under reflux for 16 h. The cooled

reaction mixture was diluted with H_2O, an aqueous solution of $NaHCO_3$ was added and the mixture was extracted with Et_2O (3x). The combined organic layers were dried (Na_2SO_4) and the solvent was removed to obtain 3.12 g (99%) of a colorless oil identified as ethyl (2*E*,4*E*)-5-(1,3-dioxolan-2-yl)-3-methylpenta-2,4-dienoate **20a**. ^{1}H-NMR (400 MHz, C_6D_6): δ 6.29 (d, *J* = 15.8 Hz, 1H, H_4), 5.96 (dd, *J* = 15.8, 5.4 Hz, 1H, H_5), 5.82 (s, 1H, H_2), 5.23 (d, *J* = 5.4 Hz, 1H, H_6), 3.99 (q, *J* = 7.1 Hz, 2H, OCH_2CH_3), 3.53 - 3.45 (m, 2H, CH_2), 3.43 - 3.36 (m, 2H, CH_2), 2.23 (s, 3H, CH_3), 0.96 (t, *J* = 7.1 Hz, 3H, OCH_2CH_3) ppm. ^{13}C-NMR (101 MHz, C_6D_6): δ 166.3 (s), 150.8 (s), 137.2 (d), 131.8 (d), 121.7 (d), 103.4 (d), 65.0 (t, 2x), 59.8 (t), 14.3 (q), 13.7 (q) ppm. HRMS (ESI^+): Calcd. for $C_{11}H_{17}O_4$ ($[M+H]^+$), 213.1121; found, 213.1120. IR (NaCl): υ 2979 (m, C-H), 2887 (m, C-H), 1713 (s, C=O), 1616 (m), 1231 (m), 1154 (s), 772 (m) cm^{-1}. UV (MeOH): λ_{max} 253 nm.

4.8. (2E,4E)-5-(1,3-Dioxolan-2-yl)-3-methylpenta-2,4-dien-1-ol (20b)

Following the general procedure for Dibal-H reduction of esters, the reaction of ethyl (2*E*,4*E*)-5-(1,3-dioxolan-2-yl)-3-methylpenta-2,4-dienoate 20a (1.39 g, 6.59 mmol) with Dibal-H (16.5 mL, 1M in hexanes, 16.5 mmol) in THF (33.4 mL) at -78 ºC for 2h, afforded 1.11 g (99%) of a colorless oil identified as (2*E*,4*E*)-5-(1,3-dioxolan-2-yl)-3-methylpenta-2,4-dien-1-ol **20b**. ^{1}H-NMR (400 MHz, C_6D_6): δ 6.43 (d, *J* = 15.8 Hz, 1H, H_4), 5.77 (dd, *J* = 15.8, 5.9 Hz, 1H, H_5), 5.54 (t, *J* = 5.4 Hz, 1H, H_2), 5.32 (d, *J* = 5.9 Hz, 1H, H_6), 3.90 (d, *J* = 5.4 Hz, 2H, $2H_1$), 3.65 - 3.54 (m, 2H, CH_2), 3.47 - 3.41 (m, 2H, CH_2), 1.47 (s, 3H, CH_3) ppm. ^{13}C-NMR (101 MHz, C_6D_6): δ 138.8 (d), 134.1 (s), 134.0 (d), 125.3 (d), 104.4 (d), 65.0 (t, 2x), 59.2 (t), 12.4 (q) ppm. HRMS (ESI+): Calcd. for $C_9H_{15}O_3$ ($[M+H]^+$), 171.1016; found, 171.1020. IR (NaCl): υ 3500-3100 (br, O-H), 2950 (m, C-H), 2883 (m, C-H), 1386 (m), 1156 (m), 1085 (m), 1014 (m), 950 (s) cm^{-1}. UV (MeOH): λ_{max} 233 nm.

4.9. Methyl (2E,4E,6E,8E,10E,12E,14E)-4,9,13-Trimethyl-15-(2,6,6-trimethylcyclohex-1-en-1-yl)pentadeca-2,4,6,8,10,12,14-heptaenoate (methyl 10'-β-apo-carotenoate) (25).

Following the general procedure for olefin metathesis, the reaction of (1,3,3-trimethyl-2-((1*E*,3*E*,5*E*,7*E*)-3,7-dimethyldeca-1,3,5,7,9-pentaenyl)cyclohex-1-ene **12** (10 mg, 0.035 mmol), methyl (2*E*,4*E*,6*E*) and (2*E*,4*E*,6*Z*)-4-methylocta-2,4,6-trienoate **24** (35.3 mg, 0.212 mmol) and 2nd generation Hoveyda-Grubbs catalyst (4.4 mg, 0.007 mmol) in toluene (0.18 mL) at 25 ºC for 5 h, afforded, after purification by column chromatography (silica gel, 98:2 hexane/EtOAc) in order of elution, 5.5 mg (58 %) of a red solid identified as *β,β*-carotene **1**, 17.2 mg (49%) of a yellowish oil identified as starting methyl (2*E*,4*E*,6*E*) and (2*E*,4*E*,6*Z*)-4-methylocta-2,4,6-trienoate **24** and 3.4 mg (24%) of a red oil identified as methyl (2*E*,4*E*,6*E*,8*E*,10*E*,12*E*,14*E*)-4,9,13-trimethyl-15-(2,6,6-trimethylcyclohex-1-en-1-yl)pentadeca-2,4,6,8,10,12,14-heptaenoate **25**. ^{1}H-NMR (400.16 MHz, $(CD_3)_2CO$): δ 7.35 (d, *J* = 15.5 Hz, 1H, $H_{12'}$), 6.96 (dd, *J* = 13.9, 11.9 Hz, 1H, H_{15}), 6.84 (dd, *J* = 14.9, 11.5 Hz, 1H, H_{11}), 6.80 - 6.72 (m, 1H, $H_{15'}$), 6.67 (d, *J* = 11.8 Hz, 1H, $H_{14'}$), 6.43 (d, *J* = 15.0 Hz, 1H, H_{12}), 6.37 (d, *J* = 11.8 Hz, 1H, H_{14}), 6.26 (d, *J* = 15.8 Hz, 1H, H_7), 6.23 (d, *J* = 11.0 Hz, 1H, H_{10}), 6.17 (d, *J* = 16.1 Hz, 1H, H_8), 5.90 (d, *J* = 15.5 Hz, 1H, $H_{11'}$), 3.69 (s, 3H, OCH_3), 2.07 - 2.05 (m, 2H, CH_2), 2.03 (s, 3H, CH_3), 2.00 (s, 3H, CH_3), 1.96 (s, 3H, CH_3), 1.71 (s, 3H, CH_3), 1.68 - 1.57 (m, 2H, CH_2), 1.51 - 1.45 (m, 2H, CH_2), 1.03 (s, 6H, 2 x CH_3) ppm. ^{13}C-NMR (100.62 MHz, $(CD_3)_2CO$): δ 167.8 (s), 149.3 (d), 140.3 (d), 139.8 (s), 138.8 (d), 138.7 (s), 137.9 (d), 137.4 (s), 135.0 (d), 134.2 (s), 132.8 (d), 131.9 (d), 129.9 (d), 129.9 (s), 127.6 (d), 127.3 (d), 116.8 (d), 51.5 (q), 40.3 (t), 34.9 (s), 33.6 (t), 29.3 (q, 2x), 22.0 (q), 19.9 (t), 12.9 (q), 12.8 (q), 12.6 (q) ppm. HRMS (ESI^+): Calcd. for $C_{28}H_{39}O_2$ ($[M+H]^+$), 407.2945; found, 407.2954. IR (NaCl): υ 2921 (m, C-H), 2856 (m, C-H), 1714 (m, C=O), 1701 (m), 1539 (m) cm^{-1}. UV (MeOH): 429 nm.

4.10. (2E,4E,6E,8E,10E,12E,14E)-4,9,13-Trimethyl-15-(2,6,6-trimethylcyclohex-1-en-1-yl)pentadeca-2,4,6,8,10,12,14-heptaenal (10'-apo-β-carotenal) (6).

Following the general procedure for Dibal-H reduction of esters, the reaction of methyl (2*E*,4*E*,6*E*,8*E*,10*E*,12*E*,14*E*)-4,9,13-trimethyl-15-(2,6,6-trimethylcyclohex-1-en-1-yl)pentadeca-2,4,6,8,10,12,14-heptaenoate **25** (11.2 mg, 0.028 mmol) with Dibal-H (6.9 µL, 0.069 mmol, 1M in hexanes) in THF (0.138 mL) at -78 ºC for 2h, afforded, after purification by column chromatography (silica gel, gradient from 85:12:3 to 80:20:0 hexane/EtOAc/Et_3N) 10 mg of a red solid identified as (2*E*,4*E*,6*E*,8*E*,10*E*,12*E*,14*E*)-4,9,13-trimethyl-15-(2,6,6-trimethylcyclohex-1-en-1-yl)pentadeca-2,4,6,8,10,12,14-heptaen-1-ol **26** which was used without further purification.

Following the general procedure for MnO_2 oxidation of alcohols, the reaction of (2*E*,4*E*,6*E*,8*E*,10*E*,12*E*,14*E*)-4,9,13-trimethyl-15-(2,6,6-trimethylcyclohex-1-en-1-yl)pentadeca-2,4,6,8,10,12,14-heptaen-1-ol **26** (10.7 mg, 0.028 mmol) with MnO_2 (52.2 mg, 0.51 mmol) and Na_2CO_3 (54.1 mg, 0.51 mmol) in THF (0.423 mL) at 25 ºC for 2h afforded, after purification by column chromatography (silica gel, gradient from 98:0:2 to 97:3:0 hexane/EtOAc/Et_3N) 6.1 mg (58% combined yield) of a red solid identified as (2*E*,4*E*,6*E*,8*E*,10*E*,12*E*,14*E*)-4,9,13-trimethyl-15-(2,6,6-trimethylcyclohex-1-en-1-yl)pentadeca-2,4,6,8,10,12,14-heptaenal **6** which was purified by HPLC (Waters Spherisorb^{TM} 10µm CN, 10x250 mm Semipreparative column with NovaPak CN 4µm precolumn, 95:5 hexane/acetone, flow rate: 3mL/min, t_R = 12 min). ^{1}H-NMR (400.16 MHz, $(CD_3)_2CO$): δ 9.59 (d, *J* = 7.6 Hz, 1H, $H_{10'}$), 7.33 (d, *J* = 15.3 Hz, 1H, $H_{12'}$), 7.08 - 6.96 (m, 1H, H_{15} or $H_{15'}$), 6.86 (dd, *J* = 15.0, 11.4 Hz, 1H, H_{11}), 6.81 - 6.74 (m, 2H, H_{14} or $H_{14'}$ + H_{15} or $H_{15'}$), 6.44 (d, *J* = 15.0 Hz, 1H, H_{12}), 6.39 (d, *J* = 12.0 Hz, 1H, H_{14} or $H_{14'}$), 6.30 - 6.11 (m, 4H, H_7 + H_8 + H_{10} + $H_{11'}$), 2.07 - 2.04 (m, 2H, CH_2), 2.04 (s, 6H, 2 x CH_3), 2.00 (s, 3H, CH_3), 1.71 (s, 3H, CH_3), 1.66 - 1.59 (m, 2H, CH_2), 1.50 - 1.46 (m, 2H, CH_2), 1.03 (s, 6H, 2x CH_3) ppm. ^{13}C-NMR (100.62 MHz, $(CD_3)_2CO$): δ 193.7 (d), 156.9 (d), 141.7 (d), 140.7 (s), 138.7 (d), 138.7 (s), 137.9 (d), 137.6 (s), 136.1 (d), 134.7 (s), 132.6 (d), 131.9 (d), 129.9 (s), 129.9 (d), 128.1 (d), 127.8 (d), 127.6 (d), 40.3 (t), 34.9 (s), 33.6 (t), 29.3 (q, 2x), 22.0 (q), 19.9 (t), 13.0 (q), 12.8 (q), 12.70 (q) ppm. HRMS (ESI^+): Calcd. for $C_{27}H_{37}O$ ($[M+H]^+$), 377.2839; found, 377.2842. IR (NaCl): υ 2920 (m, C-H), 2852 (m, C-H), 1671 (s, C=O), 1123 (s), 968 (m) cm^{-1}. UV (MeOH): 450 nm (ε 66 400 mol^{-1} L cm^{-1}).

4.11. Purification of recombinant human ALDHs and enzymatic assay

Human ALDH1A1, ALDH1A2 and ALDH1A3 were recombinantly expressed from the pET-30 Xa/LIC vector and affinity purified onto a Ni^{2+}-NTA Chelating Sepharose^{TM} Fast Flow column (GE Heathcare). Activity assays with apo-β-carotenals were carried out using detergent-free solubilization and end-point reaction, followed by HPLC, originally devised for retinoid analysis.[42] ALDH1A1 and ALDH1A2 were assayed in 50 mM HEPES, 0.5 mM EDTA, 0.5 mM DTT, pH 8.0, while

ALDH1A3 was assayed in 50 mM HEPES, 30 mM $MgCl_2$, 5 mM DTT, pH 8.0. Concentration of 12'- and 14'-apo-β-carotenal was determined based on the corresponding molar absorption coefficient in aqueous solutions at the appropriate wavelength (ε_{410} = 24,228 $M^{-1}\cdot cm^{-1}$ and ε_{416} = 15,945 $M^{-1}\cdot cm^{-1}$, for 12'-apo-β-carotenal **5** in ALDH1A1/1A2 and ALDH1A3 reaction buffer, respectively; and ε_{397} = 9,218 $M^{-1}\cdot cm^{-1}$ and ε_{340} = 9,036 $M^{-1}\cdot cm^{-1}$ for 14'-apo-β-carotenal **4** in ALDH1A1/1A2 and ALDH1A3 reaction buffer, respectively). The reaction was started by the addition of cofactor and carried out for 15 min at 37 °C in a final volume of 0.5 mL. With the aim to measure the steady state enzymatic activity, the concentration of enzyme was kept from 50- to 100-fold lower than that of the substrate for all enzymatic assays and a saturating concentration of cofactor (0.5 mM NAD^+) was used. Reaction products were extracted with hexane/dioxane/isopropanol (50:5:1, v/v) and analyzed by an HPLC-based method.[43] The organic layer was evaporated, apo-β-carotenoids were dissolved in hexane and injected onto a NovaPak® silica gel column (4 μm, 3.9 x 150 mm, Waters) in hexane:*tert*-butyl methyl ether (96:4, v/v) mobile phase, at a flow rate of 2 mL/min using a Waters Alliance 2695 HPLC instrument. Elution was monitored at 415 nm for 12'-apo-β-carotenal **5**, 400 nm for 12'-apo-β-carotenoic acid **9** and 14'-apo-β-carotenal **4**, and 373 nm for 14'-apo-β-carotenoic acid **8**, using a Waters 2996 photodiode array detector. All compound manipulations were performed under dim or red light to prevent photoisomerization.

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6. Supplementary data

General procedures, synthesis of intermediates, spectral characterization data for the products described in the text, including copies of the NMR spectra. See DOI:

7. References Section

1. (a) Britton G, Liaaen-Jensen S, Pfander HE, Eds., *Carotenoids. Part 1A. Isolation and Analysis*, Birkhäuser, Basel, 1995; (b) Britton G, Liaaen-Jensen S, Pfander H, Eds., *Carotenoids. Part 1B. Spectroscopy*, Birkhäuser, Basel, 1995; (c) Landrum T, Ed. *Carotenoids. Physical, Chemical and Biological Functions and Properties*, CRC Press, Boca Raton, FL 2010, vol. 1.
2. Britton G, Liaaen-Jensen S, Pfander H, Eds., *Carotenoids. Vol. 4. Natural Functions*, Birkhäuser, Basel 2008.
3. Britton G, Liaaen-Jensen S, Pfander H, Eds., *Carotenoids. Volume 5. Nutrition and Health*, Birkhäuser, Basel 2009.
4. Alvarez R, Vaz B, Gronemeyer H, de Lera, AR. *Chem. Rev*. **2014**; *114*: 1-125.
5. Britton G, Liaaen-Jensen S, Pfander, H, Eds., *Carotenoids. Volume 3. Biosynthesis and Metabolism*, Birkhäuser, Basel 1998.
6. (a) von Lintig J, Vogt K. *J. Biol. Chem*. **2000**; *275*: 11915-11920; (b) Wyss A, Wirtz G, Woggon WD, Brugger R, Wyss M, Friedlein A, Bachmann H, Hunziker, W. *Biochem. Biophys. Res. Commun*. **2000**; *271*: 334-336; (c) Kiefer C, Hessel S, Lampert JM, Vogt K, Lederer MO, Breithaupt DE, von Lintig J. *J. Biol. Chem*. **2001**; *276*: 14110-14116; (d) dela Seña C, Riedl KM, Narayanasamy S, Curley RW, Schwartz SJ, Harrison EH. *J. Biol. Chem.* **2014**; *289*: 13661-13666.
7. Lobo GP, Amengual J, Palczewski G, Babino D, von Lintig J. *Biochim. Biophys. Acta (BBA) - Mol. Cell Biol. Lip*. **2012**; *1821*: 78-87.
8. (a) Hu KQ, Liu C, Ernst H, Krinsky NI, Russell RM, Wang XD. *J. Biol. Chem*. **2006**; *281*: 19327-19338; Amengual J, Widjaja-Adhi MAK, Rodriguez-Santiago S, Hessel S, Golczak M, Palczewski K, von Lintig J. *J. Biol. Chem*. **2013**; *288*: 34081-34096; (b) Sui X, Kiser PD, von Lintig J, Palczewski K. *Arch. Biochem. Biophys*. **2013**; *539*: 203-213; (c) Harrison PJ, Bugg TDH. *Arch. Biochem. Biophys*. **2014**; *544*: 105-111.
9. Amengual J, Lobo GP, Golczak M, Li HNM, Klimova T, Hoppel CL, Wyss A, Palczewski K, von Lintig J. *FASEB J*. **2011**; *25*: 948-959.
10. Palczewski G, Amengual J, Hoppel CL, von Lintig J. *FASEB J*. **2014**; *28*: 4457-4469.
11. (a) Lindqvist A, Andersson S. *J. Biol. Chem*. **2002**; *277*: 23942-23948; (b) Kowatz T, Babino D, Kiser P, K. Palczewski K, von Lintig J. *Arch. Biochem. Biophys*. **2013**; *539*: 214-222.
12. (a) Parés X, Farrés J, Kedishvili N, Duester G. *Cell. Mol. Life Sci*. **2008**; *65*: 3936-3949; (b) Ruiz FX, Porté S, Pares X, Farrés J. *Front. Pharmacol*. **2012**; *3*: 58.
13. Germain P, Chambon P, Eichele G, Evans RM, Lazar MA, Leid M, de Lera AR, Lotan R, Mangelsdorf DJ, Gronemeyer H. *Pharmacol. Rev*. **2006**; *58*: 712-725.
14. (a) Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schutz G, Umesono K, Blumberg B, Kastner P, Mark M, Chambon P, Evans RM. *Cell*, **1995**; *83*: 835-839; (b) Renaud JP, Moras D. *Cell. Mol. Life Sci*. **2000**; *57*: 1748-1769; (c) Gronemeyer H, Gustafsson JA, Laudet V. *Nature Rev. Drug Disc*. **2004**; *3*: 950-964.
15. Eroglu A, Harrison EH. *J. Lipid Res*. **2013**; *54*: 1719-1730.
16. Eroglu A, Hruszkewycz DP, Curley Jr RW, Harrison EH. *Arch. Biochem. Biophys*. **2010**; *504*: 11-16.
17. Eroglu A, Hruszkewycz DP, dela Sena C, Narayanasamy S, Riedl KM, Kopec RE, Schwartz SJ, Curley Jr. RW, Harrison EH. *J. Biol. Chem*. **2012**; *287*: 15886-15895.
18. Costabile BK, Kim YK, Iqbal J, Zuccaro MV, Wassef L, Narayanasamy S, Curley Jr. RW, Harrison EH, Hussain MM, Quadro L. *J. Biol. Chem*. **2016**; *291*: 18525-18535.
19. (a) Wang X, Penzes P, Napoli JL. *J. Biol. Chem*. **1996**; *271*: 16288-16293; (b) Zhao D, McCaffery P, Ivins KJ, Neve RL, Hogan P, Chin WW, Dräger UC. *Eur. J. Biochem*. **1996**; *240*: 15-22; (c) Yoshida A, Rzhetsky A, Hsu LC, Chang C. *Eur. J. Biochem*. **1998**; *251*: 549-557; (d) Niederreither K, Subbarayan V, Dolle P, Chambon P. *Nat. Genet*. **1999**; *21*: 444-448; (e) Grün F, Hirose Y, Kawauchi S, Ogura T, Umesono K. *J. Biol. Chem*. **2000**; *275*: 41210-41218.
20. Britton G, Liaaen-Jensen S, H. Pfander H, Eds., *Carotenoids. Part 2. Synthesis*, Birkhäuser, Basel 1996.
21. (a) Bernhard K, Mayer H. *Pure Appl. Chem*. **1991**; *63*: 35-44; (b) Nicolaou KC, Härter MW, Gunzner JL, Nadin A. *Liebigs Ann. Chem*. **1997**; 1283-1301; (c) Yamano Y, Ito M. *J. Chem. Soc. Perkin Trans. 1* **1993**; 1599-1610; (d) Yamano Y, Tode C, Ito M. *J. Chem. Soc., Perkin Trans. 1* **1995**; 1895-1904; (e) Tode C, Yamano Y, Ito M. *J. Chem. Soc., Perkin Trans. 1* **1999**; 1625-1626; (f) Yamano Y, Yoshizawa M, Ito M. *J. Nutr. Sci. Vitaminol*. **1999**; *45*: 49-62; (g) Furuichi N, Hara H, Osaki T, Mori H, Katsumura S, *Angew. Chem. Int. Ed*. **2002**; *41*: 1023-1026; (h) Kajikawa T, Okumura S, Iwashita T, Kosumi D, Hashimoto H, Katsumura S. *Org. Lett*. **2012**; *14*: 808-811.
22. Nicolaou KC, Bulger PG, Sarlah D. *Angew. Chem. Int. Ed*. **2005**; *44*: 4442-4489.
23. (a) Olpp T, Brückner R. *Angew. Chem. Int. Ed*. **2006**; *45*: 4023-4027; (b) Vaz B, Domínguez M, Álvarez R, de Lera AR. *Chem. Eur. J*. **2007**; *13*: 1273-1290; (c) Burghart J, Brückner R. *Angew. Chem. Int. Ed*. **2008**; *47*: 7664-7668; (d) Woerly EM, Cherney AH, Davis EK, Burke MD. *J. Am. Chem. Soc*. **2010**; *132*: 6941-6943; (e) Fujii S, Chang SY, Burke MD. *Angew. Chem. Int. Ed*. **2011**; *50*: 7862-7864; (f) Fontan N, Vaz B, Alvarez R, de Lera AR. *Chem. Commun*. **2013**; *49*: 2694-2696; (g) Otero L, Vaz B, Alvarez R, de Lera AR. *Chem. Commun*. **2013**; *49*: 5043-5045; (h) Vaz B, Otero L, Álvarez R, de Lera AR. *Chem. Eur. J*. **2013**; *19*: 13065-13074; (i) Woerly EM, Roy J, Burke MD. *Nat. Chem*. **2014**; *6*: 484-491.
24. (a) Nicolaou KC, Bulger PG, Sarlah D. *Angew. Chem. Int. Ed*. **2005**; *44*: 4490-4527; (b) Hoveyda AH, Zhugralin AR. *Nature* **2007**; *450*: 243-251; (c) Fürstner A. *Chem. Commun*. **2011**; *47*: 6505-6511.
25. Fürstner A. *Science* **2013**; *341*: 1229713.

26. Vougioukalakis GC, Grubbs RH. *Chem. Rev.* **2010**; *110*: 1746-1787.
27. Maj J, Morzycki JW, Rárová L, Wasilewski G, Wojtkielewicz A. *Tetrahedron Lett*. **2012**; *53*: 5430-5433.
28. Jermacz I, Maj J, Morzycki JW, Wojtkielewicz A. *Toxicol. Mech. Meth.* **2008**; *18*: 469-471.
29. (a) Wojtkielewicz A, Maj J, Morzycki JW. *Tetrahedron Lett*. **2009**; *50*: 4734-4737; (b) Wojtkielewicz A, Maj J, Dzieszkowska A, Morzycki JW. *Tetrahedron* **2011**; *67*: 6868-6875.
30. Fontán N, Domínguez M, Álvarez R, de Lera AR. *Eur. J. Org. Chem.* **2011**; 6704-6712.
31. Fontán N, Alvarez R, de Lera AR. *J. Nat. Prod.* **2012**; *75*: 975-979.
32. Kajikawa T, Iguchi N, Katsumura S. *Org. Biomol. Chem*. **2009**; *7*: 4586-4589.
33. Chatterjee AK, Choi TL, Sanders DP, Grubbs RH. *J. Am. Chem. Soc*. **2003**; *125*: 11360-11370.
34. Al-Hasani SMA, Parrish DB. *J. Agr. Food Chem*. **1967**; *15*: 943-944.
35. Fürstner A, Guth O, Düffels A, Seidel G, Liebl M, Gabor B, Mynott R. *Chem. Eur. J*. **2001**; *7*: 4811-4820.
36. (a) Grela K, Harutyunyan S, Michrowska A. *Angew. Chem., Int. Ed*. **2002**; *41*: 4038-4040; (b) Michrowska A, Bujok R, Harutyunyan S, Sashuk V, Dolgonos G, Grela K. *J. Am. Chem. Soc*. **2004**; *126*: 9318-9325; (c) Samojtowicz C, Bieniek M, Grela K. *Chem. Rev.* **2009**; *109*: 3708-3742.
37. (a) Scholl M, Ding S, Lee CW, Grubbs RH. *Org. Lett*. **1999**; *1*: 953-956; (b) Nguyen ST, Grubbs RH, Ziller JW. *J. Am. Chem. Soc*. **1993**; *115*: 9858-9859.
38. Garber SB, Kingsbury JS, Gray BL, Hoveyda AH. *J. Am. Chem. Soc.* **2000**; *122*: 8168-8179.
39. (a) Estrada AF, Youssar L, Scherzinger D, Al-Babili S, Avalos J. *Mol. Microbiol.* **2008**; *69*: 1207-1220; (b) Díaz-Sánchez V, Estrada AF, Trautmann D, Al-Babili S, Avalos J. *FEBS J*. **2011**; *278*: 3164-3176; (c) Trautmann D, Beyer P, Al-Babili S. *FEBS J*. **2013**; *280*: 3685-3696.
40. Veera Reddy P, Rabago-Smith M, Borhan B. *J. Labelled Cpd. Radiopharm*. **2002**; *45*: 79-89.
41. (a) Bernhard K, Englert G, Mayer H, Müller RK, Rüttimann A, Vecchi M, Widmer E, Zell R. *Helv. Chim. Acta* **1981**; *64*: 2469-2484; (b) Haugan JA, Liaaen-Jensen S. *Acta Chem. Scand*. **1994**; *48*: 899-904.
42. Rüegg R, Lindlar H, Montavon M, Saucy G, Schaeren SF, Schwieter U, Isler O. *Helv. Chim. Acta* **1959**; *42*: 847-853.
43. Gallego O, Belyaeva OV, Porté S, Ruiz FX, Stetsenko AV, Shabrova EV, Kostereva NV, Farrés J, Parés X, Kedishvili NY. *Biochem. J*. **2006**; *399*: 101-109.
44. Kane MA, Chen N, Sparks S, Napoli JL. *Biochem. J*. **2005**; *388*: 363-369.

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