ABSTRACT

Title of Dissertation: TOTAL SYNTHESIS OF (*3R,3'R,6'R*)-LUTEIN, (*3R,3'R*)-ZEAXANTHIN AND THEIR STEREOISOMERS

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(*3R,3'R,6'R*)-Lutein (**1**) and (*3R,3'R*)-zeaxanthin (**5**) are dietary carotenoids that are found in most fruits and vegetables. Numerous studies have shown that **1** and **5** play an important role in the prevention of age-related macular degeneration (AMD) that is the leading cause of blindness. To date, the metabolic pathways of **1** and **5** in ocular tissues of an animal model in relation to AMD have not been explored. This is primarily because of the lack of a viable method for the synthesis of **1** and **5** that can be labeled with a stable isotope. Among the eight possible stereoisomers of lutein, only **1** has been previously prepared by total synthesis in 14 steps in an overall yield of 0.5%.

The total synthesis of lutein, zeaxanthin, and their stereoisomers from (±)-α-ionone has been accomplished by a C_{15}+C_{10}+C_{15} coupling strategy. Therefore, (*3R,3'R,6'R*)-lutein (**1**, 8%), (*3R,3'S,6'S*)-lutein (**2**, 7%), (*3R,3'S,6'R*)-lutein (**3**, 6%), and (*3R,3'R,6'S*)-lutein (**4**, 7%) were each prepared in a high optical purity in 7 steps. 3-Hydroxy-α-ionylideneacetaldehyde served as a common precursor to afford luteins

1 – **4** by a much shorter synthetic sequence and a higher overall yield than that of a published method for **1**. The other four stereoisomers of lutein can be similarly prepared.

(*3R,3'R*)-Zeaxanthin (**5**, 12%) and (*3S,3'S*)-zeaxanthin (**6**, 11%) were prepared in 8 steps from (±)-α-ionone *via* 3-hydroxy-α-ionone which was transformed into 3-hydroxy-β-ionone (*3R*-**42**, 22%) and its enantiomer (*3S*-**42**, 21%), respectively. The key intermediates, *3R*-**42** and *3S*-**42** were converted into the corresponding C_{15}-Wittig salts *3R*-**16** and *3S*-**16** that were used in a double Wittig reaction with the C_{10}-dialdehyde **17** to afford **5** (98% ee) and **6** (98% ee).

Utilizing Wittig salts *3R*-**16** and *3S*-**16**, (*3R*)-β-cryptoxanthin (**135**, 8%) and (*3S*)-β-cryptoxanthin (**136**, 9%) were each prepared in optical purity of 98%.

The most important feature of the strategies presented here is its application to the total synthesis of isotopically labeled and optically pure lutein, zeaxanthin, and their stereoisomers for metabolic studies. This synthesis also provides access to the C_{15}-precursors of optically active carotenoids with 3-hydroxy-ε- and 3-hydroxy-β-end groups that are otherwise difficult to synthesize.

Total Synthesis of (*3R,3'R,6'R*)-Lutein, (*3R,3'R*)-Zeaxanthin and Their Stereoisomers

by

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AMD	age-related macular degeneration
aq.	aqueous
AREDS2	age-related eye disease study 2
9-BBN	9-borabicyclo[3.3.1]nonane
calcd	calculated
CD	circular dichroism
conc	concentrated
de	diastereomeric excess
DHA	docosahexaenoic acid
DIBAL	diisobutylaluminum hydride
DIPEA	diisopropylethylamine
DMF	*N*,*N*-dimethylformamide
ee	enantiomeric excess
EI	electron ionization
EPA	eicosapentaenoic acid
Et	ethyl
FAB	fast-atom bombardment
h	hour (s)
HPLC	high-performance liquid chromatography
Hz	Hertz
IPM	isopropenyl methyl ether
i-Pr	isopropyl
J	coupling constant
K-Selectride	potassium tri-*sec*-butylborohydride
KS-Selectride	potassium trisiamylborohydride
L-Selectride	lithium tri-*sec*-butylborohydride
M^+	molecular ion
m/z	mass to charge ratio
Me	methyl
min	minute (s)
mp	melting point
MS	mass spectrometry
NMR	nuclear magnetic resonance
N-Selectride	sodium tri-*sec*-butylborohydride
Red-Al	sodium bis(2-methoxyethoxy)aluminum hydride
R_f	retardation factor

ROS	rod outer segment
RPE	retinal pigment epithelium
r.t.	room temperature
TBHP	*tert*-butyl hydroperoxide
TBME	*tert*-butyl methyl ether
t-Bu	*tert*-butyl
THF	tetrahydrofuran
TIBA	triisobutylaluminum
TLC	thin layer chromatography
UV-vis	ultraviolet-visible spectroscopy
WHE	Wadsworth-Horner-Emmons reaction

INTRODUCTION

(*3R,3′R,6′R*)-Lutein and (*3R,3′R*)-zeaxanthin are dietary carotenoids that are present in commonly consumed fruits and vegetables.[1,2] These carotenoids accumulate in the human plasma, major organs, and ocular tissues [macula, retinal pigment epithelium (RPE), ciliary body, iris, lens] and have been implicated in the prevention of age-related macular degeneration (AMD).[3,4]

Lutein contains 3 stereogenic centers at C3, C3', and C6' positions that can result in 8 possible stereoisomers. The chemical structures of 4 of these stereoisomers are shown in Figure 1. The other 4 stereoisomers of lutein (structures not shown), are those in which the configuration at C3 is *S* while the stereochemistry at C3' and C6' remains the same as those lutein isomers shown in Figure 1. Among these 8 stereoisomers, **1** and 3'-epilutein (**3**), have been detected in human plasma, organs and ocular tissues.

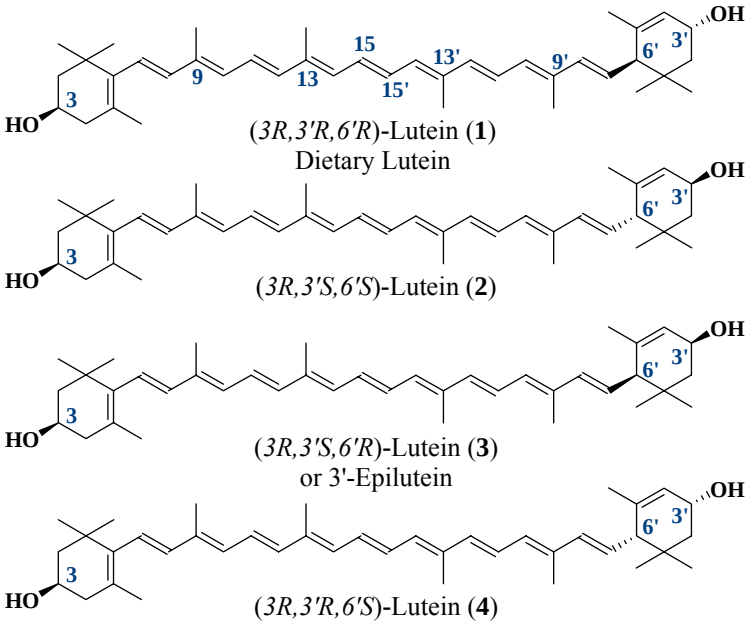


Figure 1. The chemical structure of dietary (*3R,3′R,6′R*)-lutein (**1**) and three of its stereoisomers.

Unlike lutein, zeaxanthin has a symmetrical structure and possesses two chiral centers resulting in 3 possible stereisomers (Figure 2).

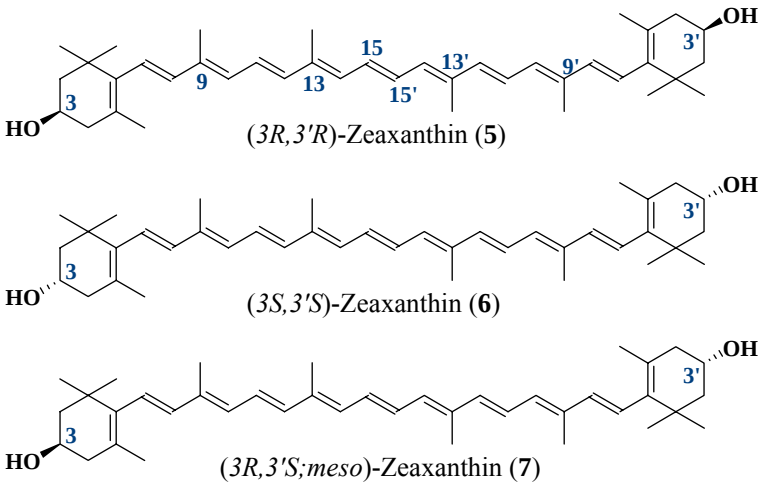


Figure 2. The chemical structures of zeaxanthin stereoisomers.

In the past decade, numerous epidemiological and experimental studies have shown that (*3R,3'R,6'R*)-lutein (**1**) and (*3R,3'R*)-zeaxanthin (**5**) play an important role in the prevention of AMD that is the leading cause of blindness in the U.S. and Western World.[5,6] Statistics from the Eye Disease Prevalence Research Group estimates that 1.8 million U.S. residents are legally blind, and another 7.3 million are at risk for vision loss from AMD.[7] The US National Eye Institute is currently conducting a multi-center, randomized clinical trial known as: "The Age-Related Eye Disease Study 2 (AREDS2)" to assess the effects of oral supplementation of (*3R,3'R,6'R*)-lutein (**1**) and (*3R,3'R*)-zeaxanthin (**5**) and/or long-chain omega-3 fatty acids (docosahexaenoic acid) [DHA] and eicosapentaenoic acid [EPA] on the progression to advanced AMD (http://www.areds2.org).

Macula is anatomically defined as an exact center of the retina and has a diameter of approximately 5.5 mm (Figure 3).

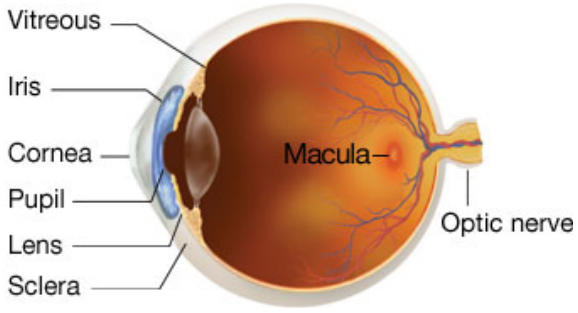


Figure 3. Anatomy of the human eye. (Source: **www.macugen.com/whatisamd.asp**)

Macula is consisted of cone photoreceptors and the initial process of vision occurs in the outer segment of these photoreceptors that provide good visual acuity (20/20). Human rod outer segment (ROS) within the macula is the region of the retina most exposed to oxidation with aging because of its relatively high oxygen tension and high concentration of long-chain polyunsaturated fatty acids (Figure 4).[7]

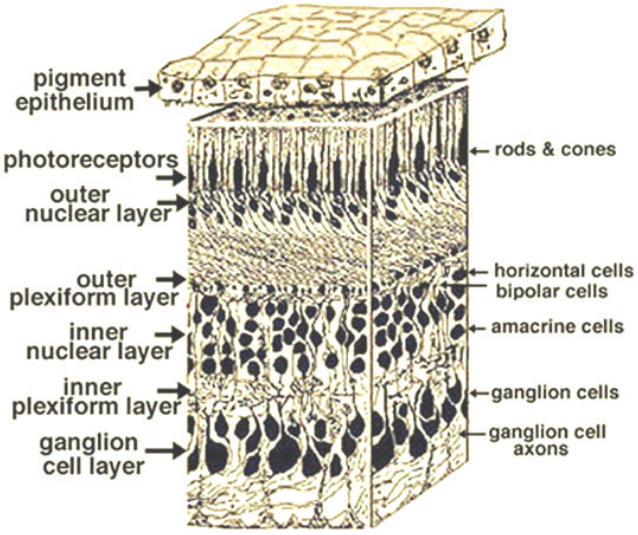


Figure 4. Three dimensional representation of a portion of human retina.

(source: http://webvision.med.utah.edu/imageswv/3dlabel.jpeg)

In 1945, Wald tentatively identified the yellow pigment in the human macula as a carotenoid belonging to the xanthophyll families in green leaves.[6] In 1985, Bone et al. presented preliminary evidence that the human macular pigment is a mixture of **1** and **5**.[8] Subsequently, in 1993 Bone et al. established the complete identification and stereochemistry of the human macular pigment as (*3R,3'R,6'R*)-lutein [(*3R,3'R,6'R*)-β,ε-carotene-3,3'-diol] (**1**), (*3R,3'R*)-zeaxanthin [(*3R,3'R*)-β,β-carotene- 3,3'-diol] (**5**), and (*3R,3'S*;*meso*)-zeaxanthin [(*3R,3'S*;*meso*)-β,β-carotene-3,3'-diol] (**7**).[9] It has been hypothesized that **1** and **5** protect the macula against photooxidative damage and prevent the onset of AMD that is the leading cause of blindness in persons aged 60 years or older.

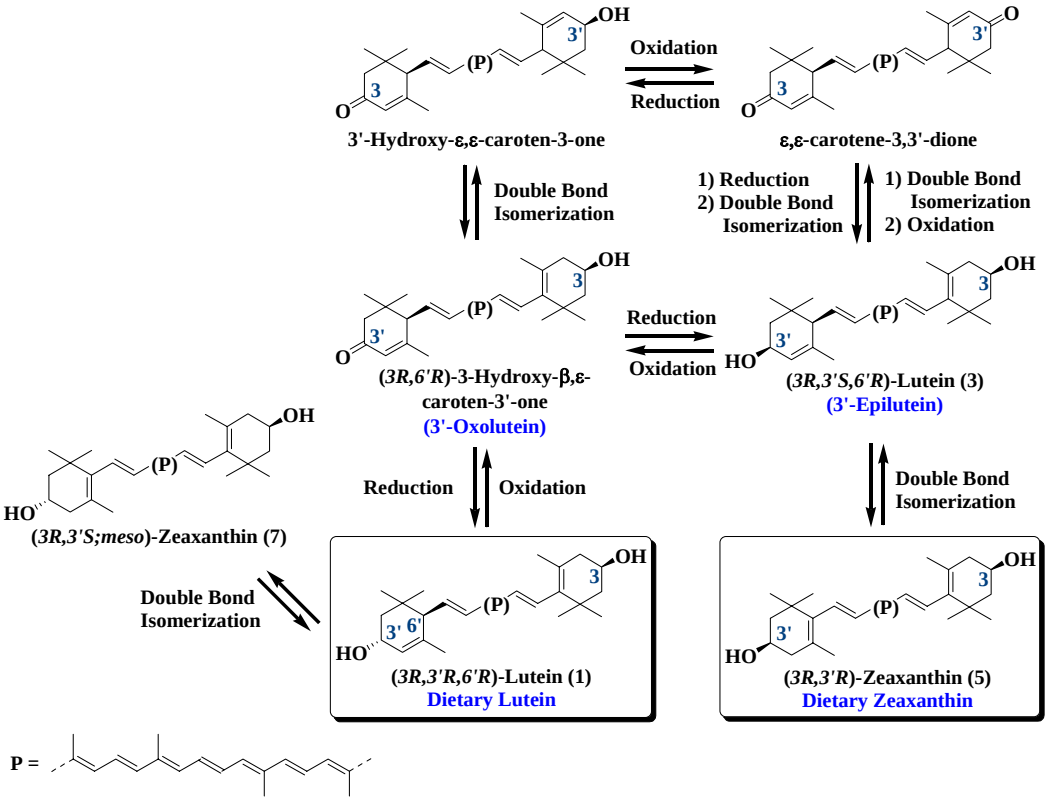
Two mechanisms for the protective role of **1** and **5** against AMD have been proposed: 1) functioning as antioxidants and/or 2) as optical filters.[10,11] In 1994, an epidemiological study concluded that persons consuming green leafy vegetables, specifically rich in **1** and **5** (ca. 6 mg/day), had 43% lower risk of AMD.[12] One of the underlying hypotheses for the protective role of carotenoids in AMD and cataract has been based on the ability of these carotenoids to act as antioxidants that can protect the human retina from photooxidation. For a review of the protective role of carotenoids against AMD, see the publication by Schalch[13] and Snodderly.[14]

Macular carotenoids are thought to function as an optical filter by absorbing short-wavelength visible light and reducing chromatic aberration.[13] This may also prevent photochemical damage to cones and retinal pigment epithelium (RPE) in the fovea which is a small depression in the center of macula.[6, 13-14] Fovea has the highest density of photoreceptors and also the highest concentration of **1** and **5**. Another

mechanism by which macular carotenoids provide protection against AMD involves their antioxidant function.[13, 15-19]

However, carotenoids are highly concentrated in the plexiform layers of the fovea where an antioxidant mechanism of action would not be expected. Rapp et al. have provided the "missing link" in this theory by detecting lutein **1** and zeaxanthin **5** in the photoreceptor rod outer segment (ROS) membranes of the human retina.[20] This region of the retina has the highest concentration of long-chain polyunsaturated fatty acids that exist in high oxygen tension and therefore most susceptible to oxidative insult.[21,22] These findings provide further support for the critical role of **1** and **5** in protecting the eye from light-induced oxidative damage.

In 1997, Khachik et al. provided preliminary evidence for the possible antioxidant role of **1** and **5** in the retina by identifying and quantifying **1**, **5**, and several of their oxidative metabolites in human and monkey retinas.[23] These metabolites were: (*3R,3'S,6'R*)-lutein (3'-epilutein) (**3**), 3-hydroxy-β,ε-caroten-3'-one (3'-oxolutein), 3'-hydroxy-ε,ε-caroten-3-one, ε,ε-carotene-3,3'-dione, and (*3R,3'S*;*meso*)-zeaxanthin (**7**). To account for the presence of these non-dietary carotenoids, Khachik et al. proposed possible metabolic transformations for dietary lutein **1** and zeaxanthin **5** in human retina as shown in Scheme 1. The authors postulated that non-dietary 3'-epilutein (**3**) and 3'-oxolutein in human ocular tissues may be formed from **1** and/or **5** as a result of a series of oxidation-reduction and double bond isomerization reactions.



Scheme 1. Proposed metabolic transformations of dietary lutein and zeaxanthin in human retina.[23]

According to these proposed metabolic transformations, allylic oxidation of **1** can result in the formation of 3'-oxolutein that may subsequently undergo stereoselective reduction to **3**. Alternatively, double bond isomerization of **5** can also result in the formation of **3**. The formation of 3'-hydroxy-ε,ε-caroten-3-one was explained by double bond isomerization of 3'-oxolutein. Similarly, double bond isomerization of **3** followed by allylic oxidation to ε,ε-carotene-3,3'-dione was proposed. With the exception of (*3R,3'S;meso*)-zeaxanthin (**7**), all of the other metabolites shown in Scheme 1 are also present in human plasma, tissues and organs at low concentrations. Therefore, the presence of these metabolites in ocular tissue can be attributed to their transport through the circulatory system or these carotenoids

may be formed locally in the eye by an independent process.[23,24,28,33-35,37] In a more recent study by Khachik et al., (*3R,3′S; meso*)-zeaxanthin (**7**) has been shown to be absent in foods, human serum and liver while relatively high concentrations of this carotenoid metabolite were found in human ocular tissues [retina, macula, retinal pigment epithelium (RPE)/choroid, ciliary body, iris, lens].[24] The presence of (*3R,3′S;meso*)-zeaxanthin (**7**) in human ocular tissue was explained by *in vivo* double bond isomerization of dietary (*3R,3′R,6′R*)-lutein (**1**).[3,4]

In 2005, Johnson et al. proved that (*3R,3′S;meso*)-zeaxanthin (**7**) was formed from dietary (*3R,3′R,6′R*)-lutein (**1**) and not from dietary (*3R,3′R*)-zeaxanthin (**5**) in a supplementation study involving xanthophyll-free monkeys.[25] In this study, the authors raised Rhesus monkeys on a diet that was free from xanthophylls [(*3R,3′R,6′R*)-lutein (**1**) and dietary (*3R,3′R*)-zeaxanthin (**5**)] and then fed these animals with either **1** or **5** supplements. (*3R,3′S;meso*)-Zeaxanthin (**7**) was only detected in the retinas of the lutein-fed monkeys after 6-8 month while this carotenoids was absent in the retinas of zeaxanthin-fed monkeys. In 2006, this was also confirmed in a study by Khachik et al. in which the authors demonstrated that (*3R,3′S;meso*)-zeaxanthin levels in the retinas, ciliary body, and lens of Rhesus Macaques is substantially increased with high dose (10 mg per kg body weight/day) supplementation with **1** after 1 year.[4]

While the experimental evidence to date unequivocally supports the conversion of dietary lutein **1** to *meso*-zeaxanthin (**7**) in the human ocular tissues, the origin of the other metabolites is not known at present. It is quite likely that dietary lutein **1** and zeaxanthin **5** in the eye undergo oxidation, reduction and double bond isomerization

reactions by photo-induced and/or enzymatic transformation to their metabolites. However, the nature of these reactions and the enzymes that may be involved remains unclear.

The proposed metabolic transformation of **1** and **5** shown in Scheme 1 can be best compared to the ocular metabolism of retinol that also involves oxidation-reduction and isomerization reactions. For example, if **1** and **5** act as antioxidants to protect the macula from oxidative injury, it would be reasonable to assume that at the early stage of AMD, the normal metabolic cycle of these carotenoids would be adversely affected. These metabolic transformations are most likely to involve certain proteins and enzymes. In such a case, characterization of the enzymes and proteins whose improper function may be responsible for the defective metabolism of **1** and **5** at the onset of AMD is essential. Unlike the metabolic cycle of vitamin A in the eye that has been well established, enzymes and proteins that may be involved in the metabolic transformations of **1** and **5** in the eye are yet to be identified. The characterization of these enzymes and proteins and understanding of their function in the human eye could provide a significant breakthrough in preventing AMD. However, prior to isolation and characterization of the enzymes and proteins that may be involved, the metabolic cycle of **1** and **5** needs to be fully established. For example, allylic oxidation of **1** at C3' that results in the formation of 3'-oxolutein may involve an oxido-reductase enzyme similar to the allylic oxidation of retinol to retinaldehyde (retinal) and the reverse reduction by retinol dehydrogenase.

Due to the invasive nature, metabolic studies that investigate such transformation would have to be conducted in an appropriate animal model. Rhesus Masques have

been shown to serve as the ideal animal model in supplementation studies with **1** and **5**.[26] Japanese quail (*Cotornix japonica*) have also been identified as a suitable non-primate animal model due to the distribution of carotenoids and their metabolites in their retinas that is similar to that of humans.[27]

Regardless of the selection of the animal model, the proposed metabolic pathways shown in Scheme 1 need to be investigated in supplementation studies with isotopically labeled **1** and **5**. In exploring biochemical and metabolic transformations, radiolabeled compounds (^{3}H, ^{14}C) are normally employed. However, in the case of carotenoids, the total synthesis of these compounds involves numerous steps and would require large amounts of radioactive water or carbon-14 source that present serious hazards with respect to handling and waste disposal. Consequently, stable isotopes of lutein **1** and zeaxanthin **5** would be ideal for metabolic studies with these compounds. In selection of the stable isotope (^{13}C or ^{2}H), two major factors need to be considered, these are: 1) no scrambling or exchange of the label with other positions in the molecule should occur, and 2) no isotope dilution as a result of exchange of label with solvent and/or reagents should take place.

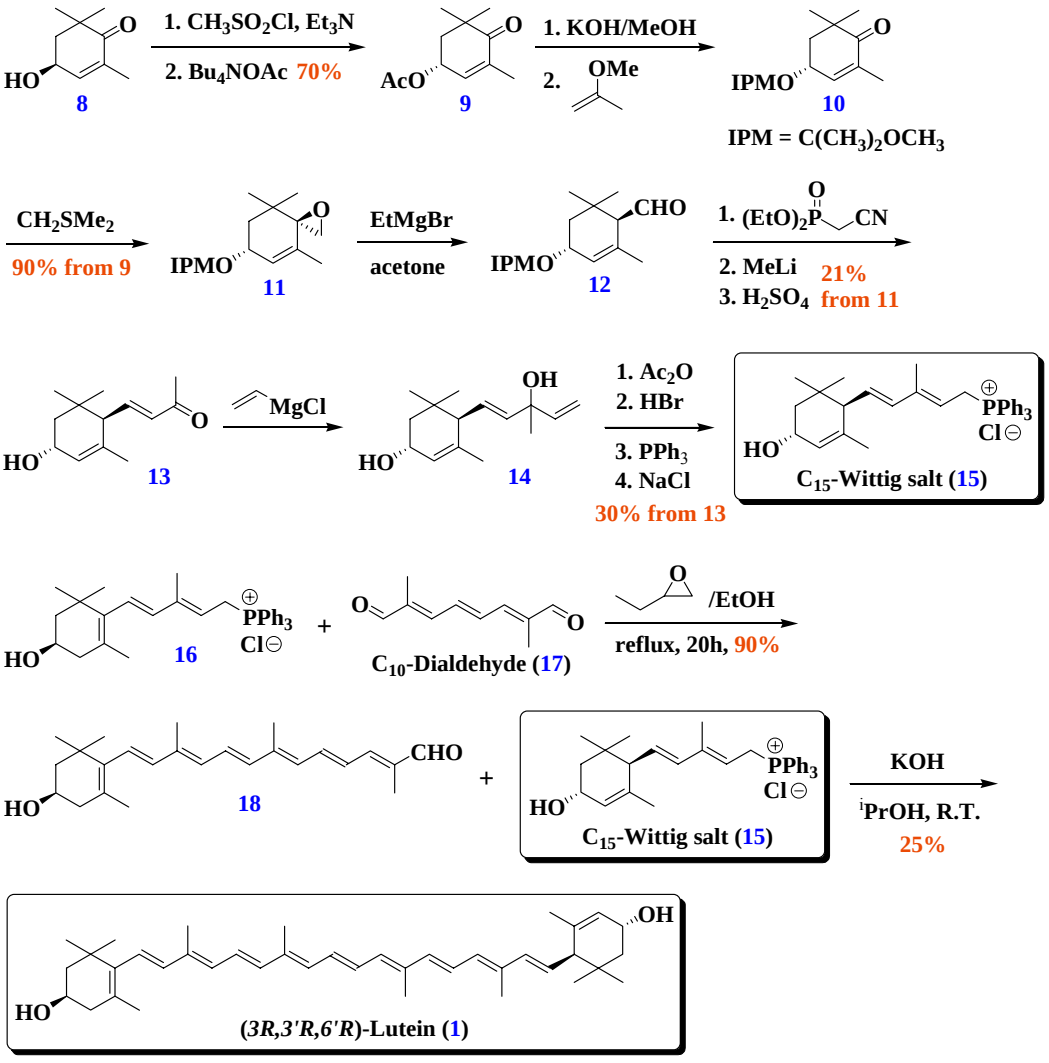
Several carotenoids specifically labeled with deuterium have been synthesized and used in the study of their fragmentation patterns in mass spectrometry (MS).[28-30] Comparison of the fragmentation patterns of ^{2}H- and ^{13}C-labeled carotenoids, however, has shown that ^{13}C-labeled carotenoids are the material of choice since no scrambling or the exchange of label is observed during their fragmentation.[31] Consequently, MS fragmentation patterns and mechanisms can be elucidated unambiguously. In metabolic studies with **1** and **5**, where certain transformations such

as oxidation-reduction and double bond isomerization reactions (Scheme 1) are of particular interest, the location of the ^{13}C-label is not crucial. This is because the general skeleton of these carotenoids remains unchanged throughout these metabolic reactions.[3,27,32-35] However, in metabolic studies with (*3R,3'R,6'R*)-lutein (**1**) and (*3R,3'R*)-zeaxanthin (**5**), it is imperative that at least four carbons in the molecules of these carotenoids are labeled. This is based on the fact that the metabolites of **1** and **5** are either isomeric to these carotenoids or are only two mass units lower than their parent compounds (Scheme 1). Therefore by increasing the molecular weight in the labeled compound by four mass units, one can readily distinguish the metabolites of **1** and **5** from their unlabelled counterparts.

With the wealth of information on the distribution of ocular carotenoids and their metabolites in humans, monkeys, and quail, metabolic studies with carotenoids labeled with C-13 would be expected to be relatively straightforward. However, to date the metabolism of **1** and **5** in the ocular tissues of an appropriate animal model in relation to AMD has not been explored. This is primarily because of the lack of an efficient and economically viable method for the synthesis of **1** and **5** that can be labeled with a stable isotope. The major difficulty with the total synthesis of (*3R,3'R,6'R*)-lutein (**1**) is due to the presence of 3 stereogenic centers mentioned at the beginning of this dissertation that can result in 8 possible stereoisomers (Figure 1).

At present, it is not known whether the other lutein stereoisomers could be formed in humans as a consequence of metabolic transformation of **1**. Therefore, the fate and biological activity of other lutein stereoisomers in humans remains unexplored due to the lack of availability of these carotenoids. While **5** has been

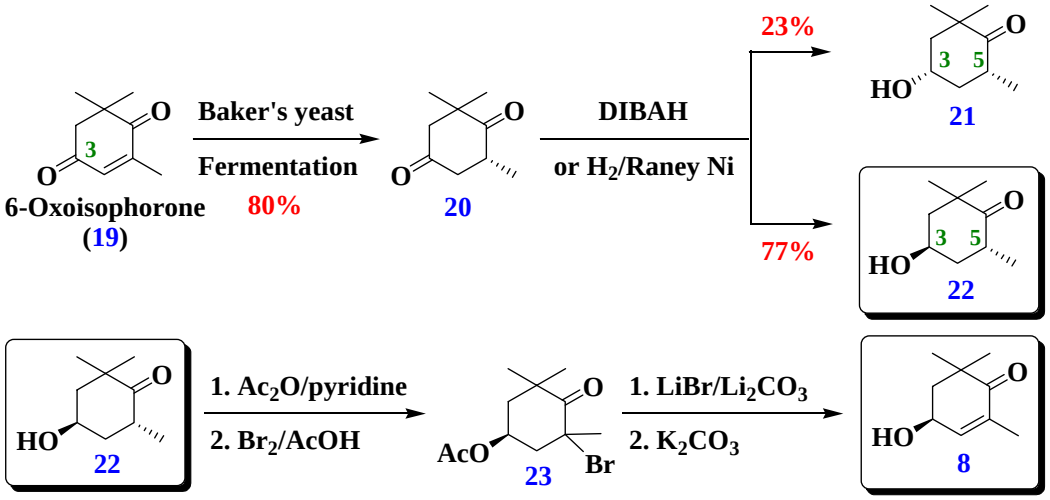
commercially available by total synthesis for more than two decades, the industrial production of **1** and its stereoisomers by chemical synthesis has not yet materialized. Consequently, **1** is commercially produced from saponified extracts of marigold flowers.[36] The total synthesis of (*3R,3'R,6'R*)-lutein (**1**) has been reported by Mayer and Rüttimann in 1980 who employed C_{15}+C_{10}+C_{15} double Wittig coupling strategy to prepare this carotenoid in an overall yield of 1% (Scheme 2).[37]



Scheme 2. Total synthesis of (*3R,3'R,6'R*)-lutein (**1**) according to Mayer and Rüttimann.[37]

Reaction of **8** with mesyl chloride gave the corresponding mesylate which, on treatment with tetrabutylammonium acetate, was converted into the acetoxyketone **9** with complete inversion of configuration. The introduction of the second chiral center was achieved by epoxidation of the protected hydroxyketone **10** with dimethyl sulphonium methylylide that led to the isolation of epoxide **11**. The product of the reaction of the EtMgBr with acetone was then allowed to catalyze stereoselective ring opening reaction of epoxide **11** to yield the protected hydroxyl-α-cyclocitral **12**. The authors did not provide any rationale for the stereoselectivity in this reaction and the possible formation of the other stereoisomers of **12** was not discussed. Subsequent chain lengthening by Wadsworth-Horner-Emmons (WHE) reaction gave a nitrile, which on treatment with MeLi, was converted into C_{13}-hydroxyketone **13**. Vinylation of **13** gave a mixture of epimeric vinyl carbinols **14** which were treated with PPh_3 and aqueous HBr to yield the corresponding C_{15}-phosphonium bromide salt. Since the bromide salt was not stable, it was converted to the chloride salt with aqueous NaCl to afford **15**.

The Wittig reaction of **16** that was previously prepared by the authors for the synthesis of (*3R,3'R*)-zeaxanthin (**5**) with commercially available 2,6-dimethyl-octa-2,4,6-triene-1,8-dial (C_{10}-dialdehyde **17**)[38] gave the C_{25}-hydroxyaldehyde **18** (Scheme 2). The final step of the synthesis of **1** involved the Wittig reaction of aldehyde **18** with Wittig salt **15**. The key starting material for this synthesis, synthon **8**, had to be prepared from 6-oxoisophorone in 3 steps in an overall yield of 25% (Scheme 3).[39] Therefore, the overall yield of the total synthesis of **1** according to Mayer and Rüttimann is less than 1%.



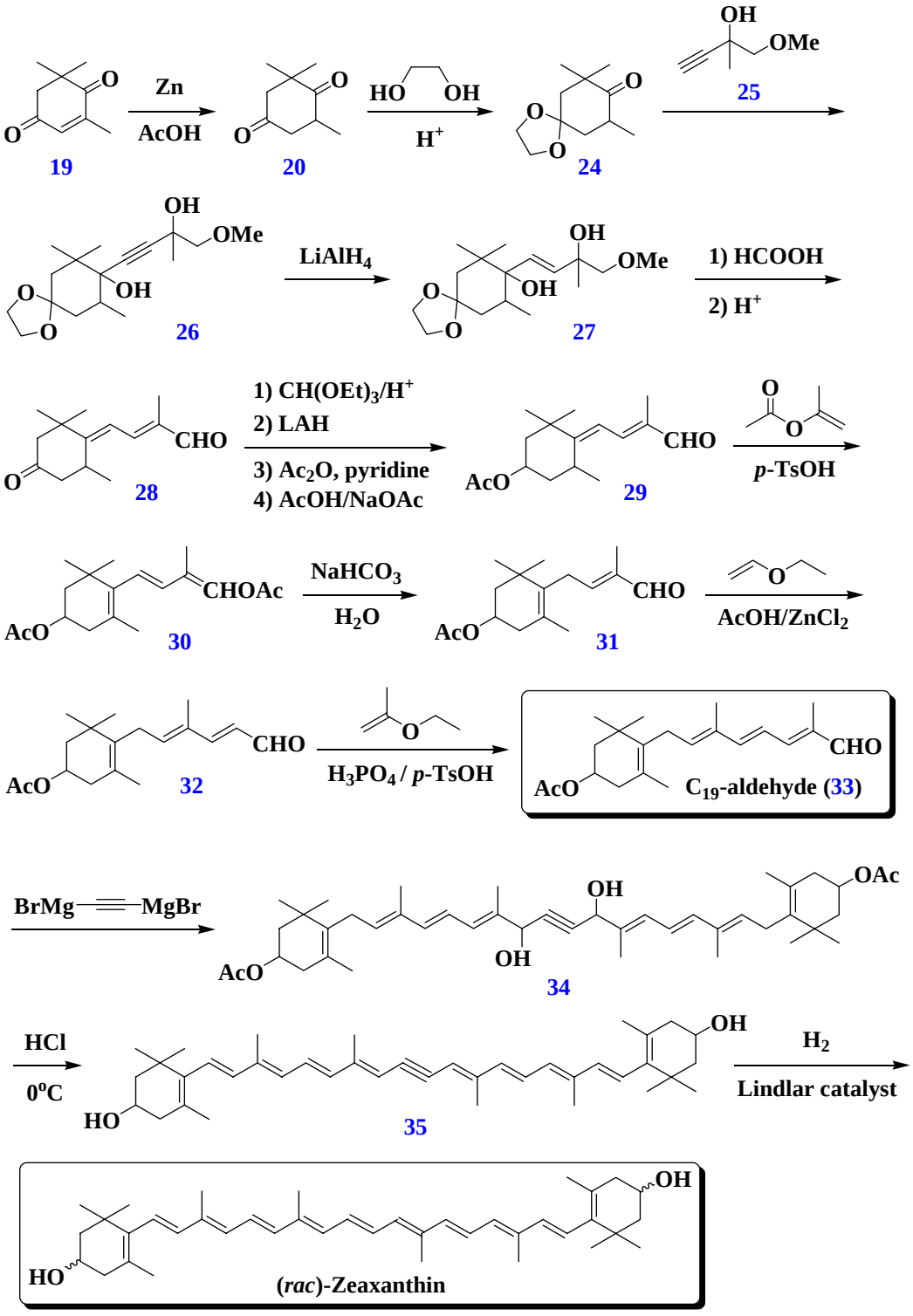
Scheme 3. Preparation of the hydroxylketone **8** from 6-oxoisophorone (**19**).[39]

The chiral ketone **8** was synthesized in three steps starting from the enantiomeric fermentative reduction of 6-oxoisophorone (**19**) using Baker's yeast to yield the saturated chiral diketone **20**. Reduction of this chiral diketone by hydrogenation over Raney Ni followed by diastereomeric separation afforded the chiral hydroxyketone **22**. After protection of the hydroxyl group, this chiral hydroxyketone was brominated under acidic condition to yield **23** which was dehydrobrominated to **8.** Consequently, this synthetic approach to (*3R,3'R,6'R*)-lutein (**1**) from 6-oxoisophorone (**19**) did not provide an efficient and economically viable route for industrial production of this carotenoid. To date, this remains as the only reported synthesis of **1** and no attempt has been made to prepare the other stereoisomers of this carotenoids by total synthesis. However, it should be noted that Khachik has reported the partial synthesis of 3'-epilutein (**3**) from **1**.[40] Thus, the main objective of the research presented in this dissertation was to develop viable processes that could be applied to the synthesis of isotopically labeled lutein, zeaxanthin, and their stereoisomers.

Unlike **1**, zeaxanthin (**5**) has a symmetrical structure and possesses two chiral centers resulting in 3 possible stereoisomers and is much easier to synthesize. The first total synthesis of optically inactive (±)-zeaxanthin was reported in 1957 by Isler et al. employing C_{19}+C_2+C_{19} Grignard coupling strategy (Scheme 4).[41]

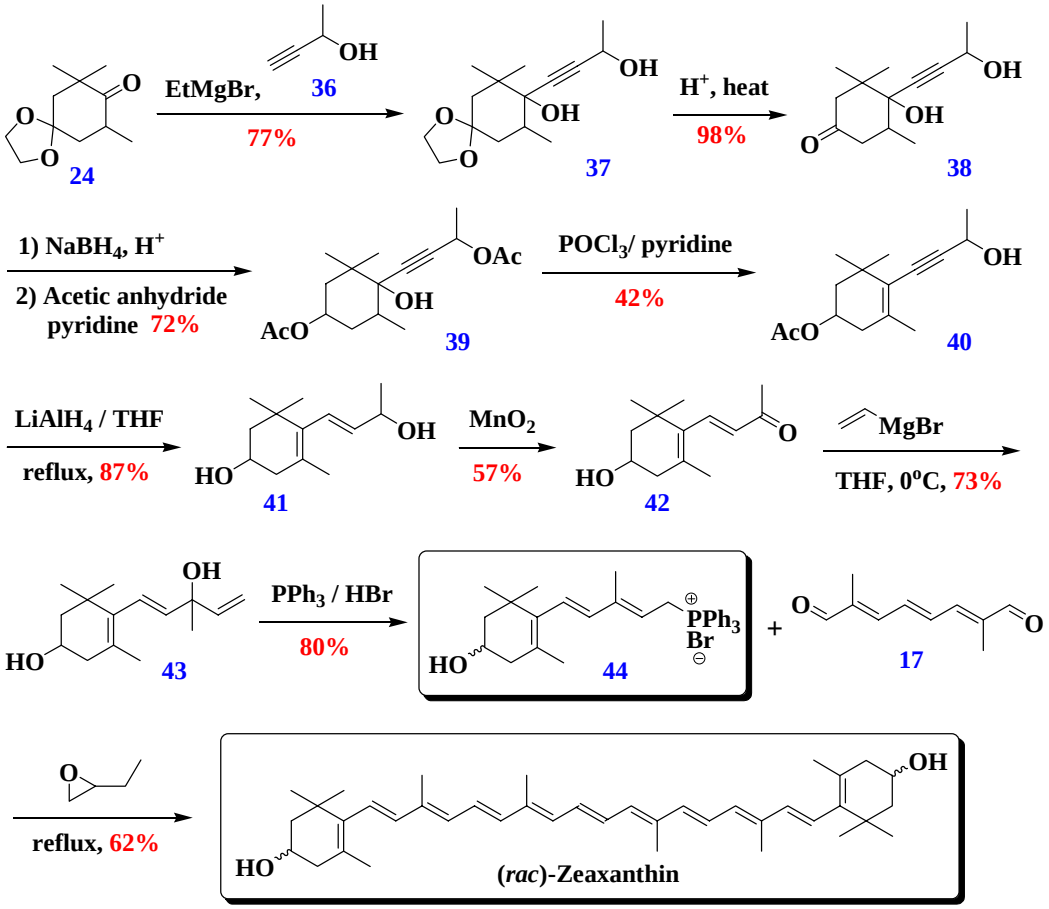
6-Oxoisophorone (**19**) was reduced chemically under acidic condition to racemic saturated diketone **20**. Unlike the stereoselective reduction of 6-oxoisophorone (**19**) discussed earlier, optically pure isomers of diketone **20** was not needed for this synthesis. The less sterically hindered keto group in **20** was selectively protected and 4-ethylenedioxy-2,2,6-trimethylcyclohexanone (**24**) was then coupled with the C_5-acetylenic synthon **25** to give **26** (Scheme 4). The triple bond of **26** was reduced with LAH to **27** and the ketal **27** was simultaneously deprotected and dehydrated to C_{14}-aldehyde **28** under acidic condition. This aldehyde was then further transformed into **29** by the following sequence of reactions: selective protection of the aldehyde group, LAH reduction of the ketone functionality, acetylation of the resulting hydroxyl group, and deprotection of the aldehyde group. Isomerization of **29** to **31** was accomplished via the corresponding enol acetate **30**. In the following step, **31** was transformed into C_{19}-aldehyde **33** via **32** by two consecutive enol ether condensations.

The final coupling of two of this C_{19}-synthon with acetylene dimagnesium bromide gave 3,3'-diacetoxy-β-C_{40}-diol (**34**) (Scheme 4). Dehydration of (**34**) with hydrochloric acid gave 15,15'-dehydrozeaxanthin (**35**) which upon hydrogenation with Lindlar catalyst afforded the desired (±)-zeaxanthin. However, the overall yield of this synthesis was not stated in this publication.



Scheme 4. Synthesis of (±)-zeaxanthin by Isler et al.[41]

In 1971, a more practical total synthesis of (±)-zeaxanthin was reported by Loeber et al. by implementing a C_{15}+C_{10}+C_{15} Wittig coupling reaction as shown in Scheme 5.[42]

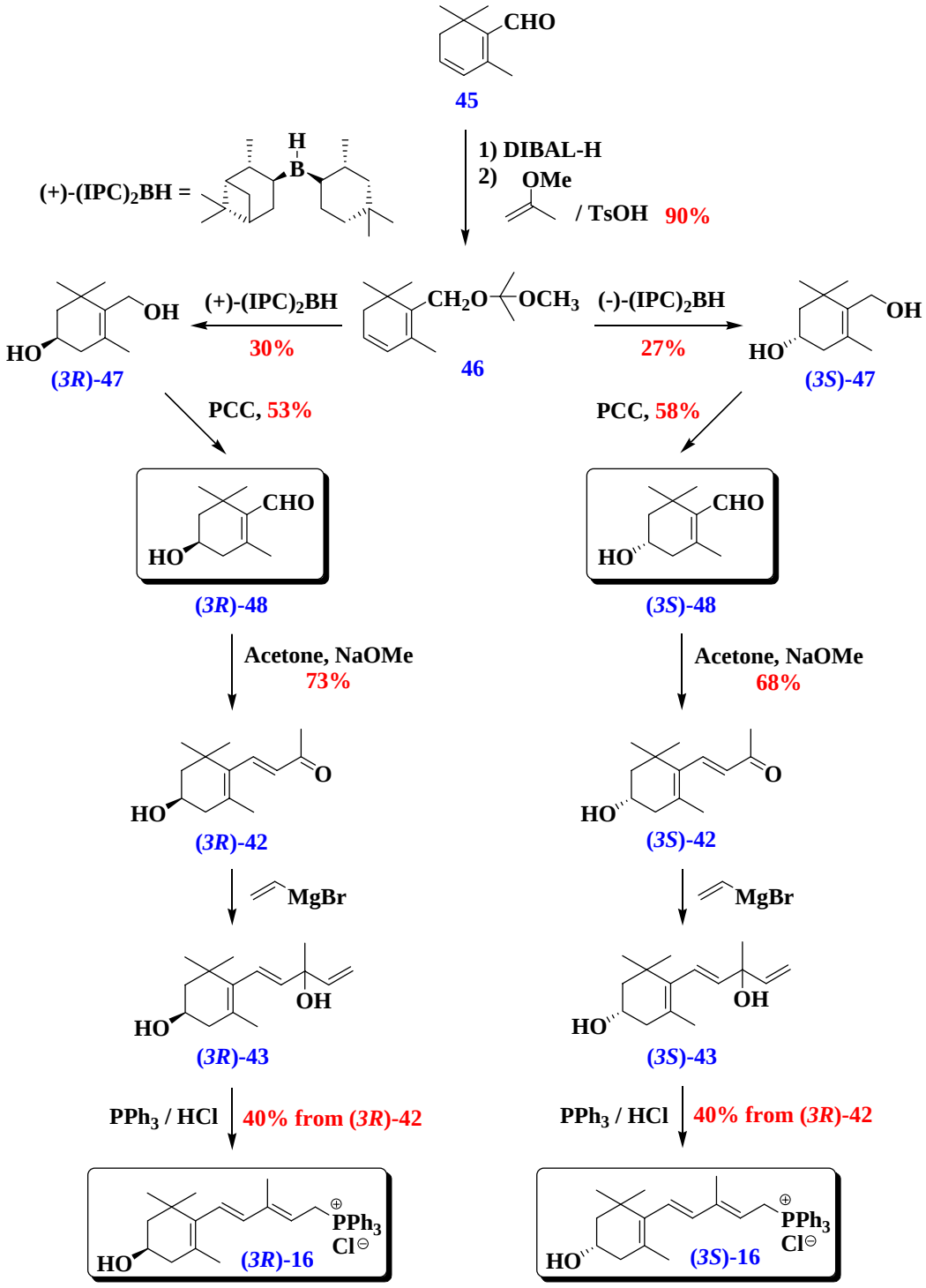


Scheme 5. The total synthesis of (±)-zeaxanthin according to the method of Loeber et al.[42]

The key intermediate for this synthesis was (±)-3-hydroxy-(β-ionylideneethyl)-triphenylphosphonium bromide (**44**) that was coupled with the commercially available C_{10}-dialdehyde **17** in a double Wittig reaction to yield a racemic mixture of zeaxanthin. This (±)-C_{15}-Wittig salt **44** was prepared in 8 steps from protected ketone (**24**) that was sequentially converted to 3-hydroxy-vinyl-α-ionol (**43**) in 6.6% yield.

The reaction of ketone **24**, prepared from 6-oxoisophorone (**19**), with the Grignard reagent prepared from but-3-yn-2-ol (**36**) afforded glycol **37** that was deprotected to dihydroxyketone **38**. In the following step, **38** was reduced with $NaBH_4$ and the resulting triol was protected to **39** which was dehydrated with phosphorus oxychloride to yield enyne **40**. Partial reduction of the acetylenic bond in **40** with $LiAlH_4$ in THF gave 3-hydroxy-β-ionol (**41**); this was subsequently oxidized with MnO_2 to 3-hydroxy-β-ionone (**42**). Reaction of this β-ionol **42** with vinyl magnesium bromide led to vinyl alcohol **43** that was transformed into the racemic Wittig salt **44**. The final coupling of the Wittig salt **44** with the C_{10}-dialdehyde **17** gave (±)-zeaxanthin in an overall yield of 4.1%.

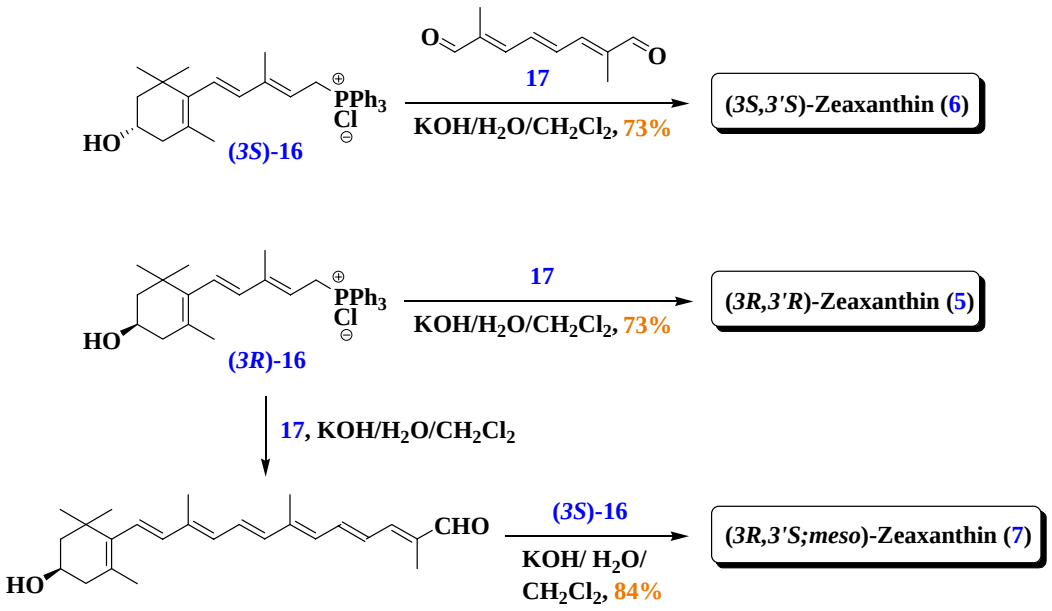
The first total synthesis of optically active (*3R)*-3-hydroxy-(β-ionylideneethyl)-triphenylphosphonium chloride [**(*3R*)-16**] and its (*3S)*-isomer [**(*3S*)-16**] was reported in 1980 by Rüttimann and Mayer (Scheme 6).[43] The key starting materials in this synthesis were the chiral aldehydes, (*3R)*-3-hydroxy-β-cyclogeranial [**(*3R*)-48**] and (*3S)*-3-hydroxy- β-cyclogeranial [**(*3S*)-48**], that were each prepared in 5 steps from safranal (**45**).



Scheme 6. Synthesis of (*3R*)-3-hydroxy-(β-ionylideneethyl)triphenylphosphonium chloride and its (*3S)*-isomer reported by Rüttimann and Mayer.[43]

The aldehyde **45** was first reduced with DIBAL-H and was subsequently protected as isopropenyl methyl ether to give the protected safranal (**46**). In the following step, **46** was seperately hydroborated with (+)-diisopinocampheylborane [(+)-$(IPC)_2BH$] and (–)-diisopinocampheylborane [(–)-$(IPC)_2BH$] to yield chiral diols **(*3R*)-47** and **(*3S*)-47**, respectively. These alcohols were separately oxidized to their corresponding chiral aldehydes **(*3R*)-48** and **(*3S*)-48** which were elongated to (*3R*)-3-hydroxy-β-ionone [**(*3R*)-42**] and its *3S*-enantiomer [**(*3S*)-42**] by aldol condensation with acetone, respectively.

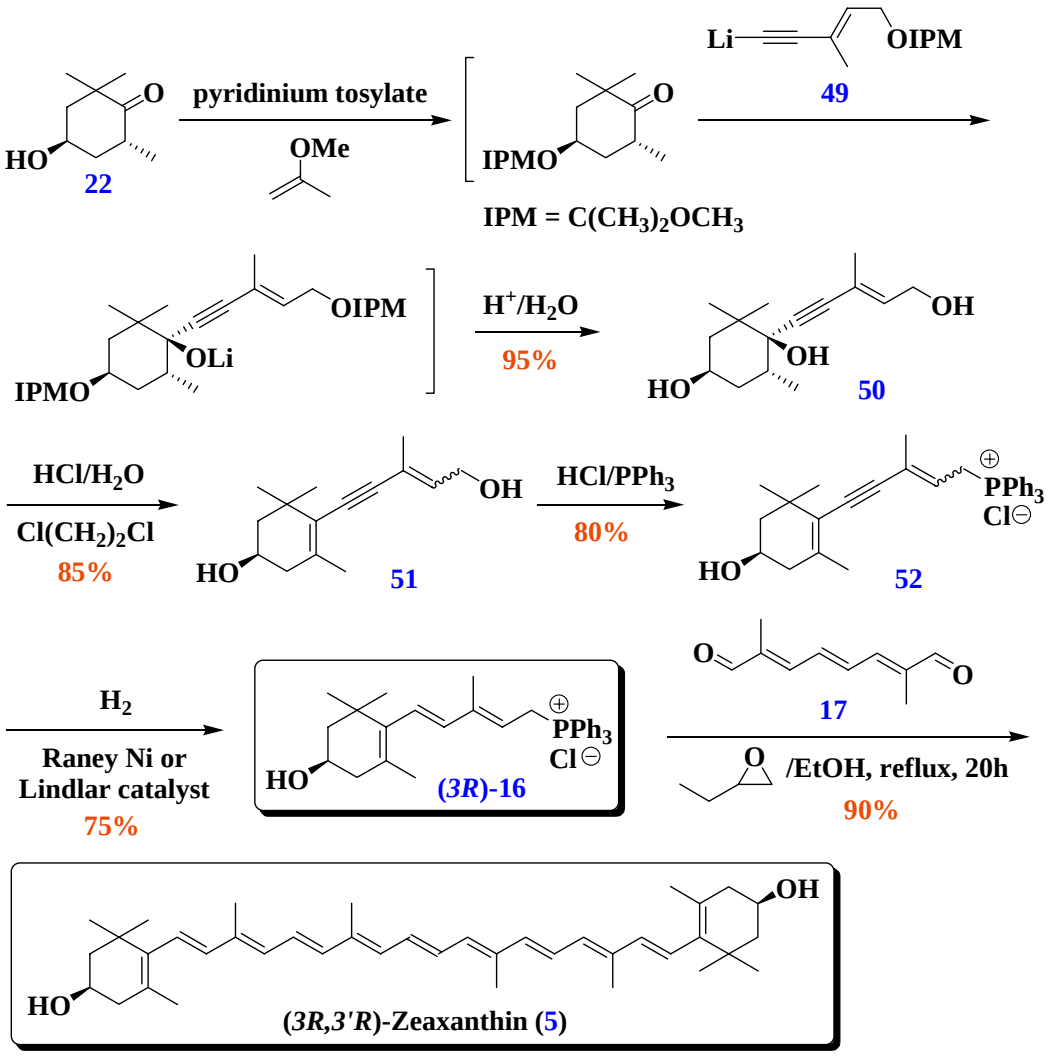
The method of Loeber et al. that was described earlier in Scheme 5 was employed to transform hydroxyionones **(*3R*)-42** and **(*3S*)-42** into their corresponding C_{15}-Wittig salts **(*3R*)-16** and **(*3S*)-16**, respectively.[42] These optically active Wittig salts were then transformed into (*3R,3'R*)-zeaxanthin (**5**), (*3S,3'S*)-zeaxanthin (**6**), and (*3R,3'S;meso*)- zeaxanthin (**7**) in double Wittig reactions with C_{10}-dialdehyde **17** (Scheme 7).[42]



Scheme 7. Transformation of (*3R*)-16 and (*3S*)-16 into zeaxanthin **5**, **6**, and **7**.

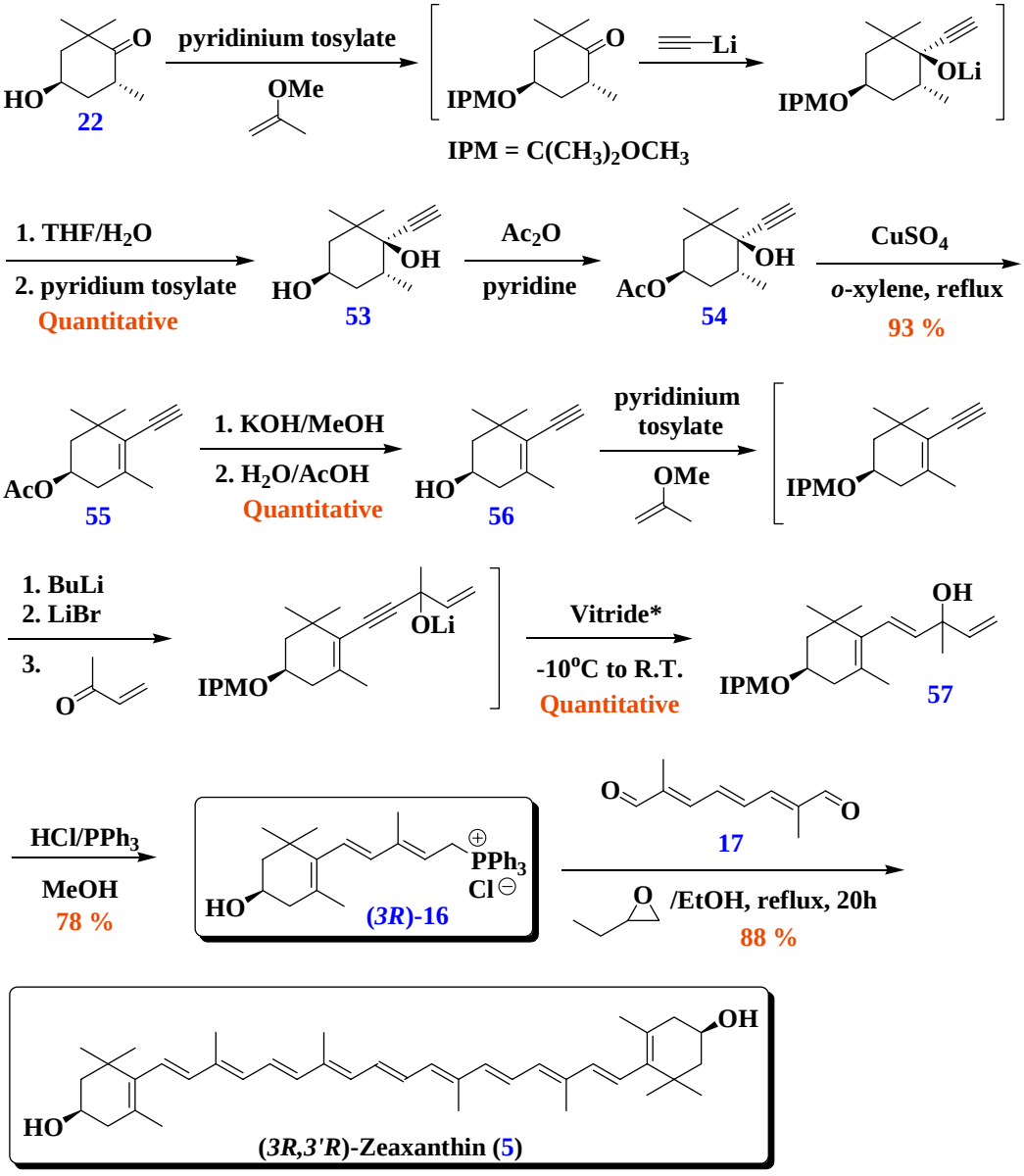
In 1990, two additional processes for the technical synthesis of (*3R,3'R*)-zeaxanthin *via* Wittig salt **(*3R*)-16** were also reported by Widmer et al.[44] and Soukup et al.[45] These processes did not involve 3-hydroxy-β-ionone as an intermediate but started from the readily available, optically active (*4R*)-4-hydroxy-2,2,6-trimethylcyclohexanone (**22**) which had been previously prepared from 6-oxoisophorone (**19**) in 62% yield by Mayer and Rüttimann for the synthesis of lutein (**1**). In the first process, the C_{15}-Wittig salt, (*3R)*-3-hydroxy-(β-ionylideneethyl)triphenylphosphonium chloride [**(*3R*)-16**] was synthesized from **22** by a C_9+C_6 coupling strategy as shown in Scheme 8.

The hydroxyl group of **22** was first protected with isopropenyl methyl ether and the resulting ketone was then coupled with C_6-synthon, (*E*)-3-methylpent-2-en-4-yn-1-ol (**49**); after acidic work-up, triol **50** was obtained in 95% yield. The dehydration of **50** was carried out with aqueous HCl in 1,2-dichloroethane in two phase to give diol **51** which was converted to the acetylenic Wittig salt **52**. After partial hydrogenation of **52** with Lindlar catalyst or Raney Ni, Wittig salt **(*3R*)-16** was obtained in an overall yield of 30% from **19**. In the final coupling step, Widmer et al. employed C_{15}+C_{10}+C_{15} strategy that had been previously developed by Loeber et al. to prepare (*3R,3'R*)-zeaxanthin (**5**) in an overall yield of 27%.



Scheme 8. The technical synthesis of (*3R,3′R*)-zeaxanthin (**5**) by Widmer et al.[44]

In the second technical synthesis of optically active (*3R,3′R*)-zeaxanthin, Soukup et al. employed a different synthetic strategy to improve the over yield from 27% to 39% (Scheme 9).[45] The hydroxyl group of **22** was first protected and the resulting ketone was allowed to react with Li-acetylide to yield diol **53**. After acetylation with Ac_2O, the monoacetate **54** was dehydrated with $CuSO_4$ in refluxing *o*-xylene to **55**; this was followed by saponification to yield enyne **56**.



*Vitride = $NaAlH_2(OCH_2CH_2OCH_3)_2$ [Sodium bis(2-methoxyethoxy)aluminum dihydride solution]

Scheme 9. The technical synthesis of (*3R,3'R*)-zeaxanthin (**5**) by Soukup et al.[45]

The acetylenic alcohol **56** was first protected as isopropenyl ether and was subsequently elongated with methyl vinyl keone to protected vinyl-β-ionol **57** via a three-step sequence carried out as a one-pot procedure. β-Ionol **57** was then transformed into Wittig salt **(*3R*)-16**. Therefore, the technical synthesis of zeaxanthin

5 was accomplished in 11 steps in an overall yield of 39% and is currently used in industrial production.

In summary, since 1971 numerous reports have clearly demonstrated that the synthesis of (±)-zeaxanthin and its three stereoisomers can be readily accomplished from the Wittig reaction of the racemic or optically active C_{15}-Wittig salt **(*3R*)-16** and **(*3S*)-16** with C_{10}-dialdehyde by a C_{15}+C_{10}+C_{15} coupling strategy. Furthermore, in the synthetic strategies developed by Loeber et al. as well as Rüttimann and Mayer, 3-hydroxy-β-ionone (**42**) has been shown to serve as the key starting material for the total synthesis of zeaxanthin. However, the synthesis of optically active (*3R*)-**42** and its (*3S*)-isomer by a relatively straightforward process is lacking and the development of such a process can considerably simplify the total synthesis of zeaxanthin and their stereoisomers.

According to the synthetic strategies described here, it is apparent that while zeaxanthin can be prepared in a good overall yield, the total synthesis of lutein is much more challenging. Therefore the objectives of the research presented here were: 1) to employ a convergent synthetic strategy to prepare the end-groups of luteins **1** – **4** and zeaxanthins **5** – **7** with appropriate stereochemistry from a commercially available and inexpensive achiral precursor and 2) to employ these end-groups to develop efficient total syntheses of unlabeled optically active **1** – **7** that can be applied to the synthesis of carotenoids labeled with carbon-13 for future metabolic studies.

NOMENCLATURE

Carotenoid numbering system has been used for compounds **73** – **84** to allow comparison of their stereochemical transformations to compounds **1** – **4** and **64** – **71**. The correct systematic names of these carotenoid precursors followed by their common names are shown in brackets as follows: **73** – **76**, (*2E,4E*)-3-methyl-5-(2,6,6-trimethyl-4-hydroxy-2-cyclohexen-1-yl)penta-2,4-dienal [(*7E,9E*)-3-hydroxy-α-ionyl-ideneacetaldehyde]; **77** – **80**, (*2E,4E*)-3-methyl-5-(2,6,6-trimethyl-4-hydroxy-2-cyclohexen-1-yl)penta-2,4-dienenitrile [(*7E,9E*)-3-hydroxy-α-ionylideneacetonitrile]; **81**: (*2E,4E*)-, **82**: (*2Z,4E*)-3-methyl-5-(2,6,6-trimethyl-4-oxo-2-cyclohexen-1-yl)penta-2,4-dienenitrile, [**81**: (*7E,9E*)-, **82**: (*7E,9Z*)-3-keto-α-ionylideneacetonitrile]; **83**: (*2E,4E*)-, **84**: (*2Z,4E*)-3-methyl-5-(2,6,6-trimethyl-2-cyclohexen-1-yl)penta-2,4-dienenitrile, [**83**: (*7E,9E*)-, **84**: (*7E,9Z*)-α-ionylideneacetonitrile].

RESULTS AND DISCUSSION

The chemical structure of carotenoids is consisted of a central polyene chain that is attached to two end groups on each side. One of the most challenging tasks in the total synthesis of carotenoids is to construct the polyene chain of these molecules with an (*all-E*)-stereochemistry. This is because the *all-E*-isomers of carotenoids are crystalline whereas their *Z*-stereoisomers are difficult to crystallize. In an unsymmetrical molecule such as (*3R,3'R,6'R*)-lutein (**1**) numerous mono-*Z* and di-*Z*-isomers are possible. However, *7Z*, *7'Z*, *11Z*, and *11'Z*-bonds are sterically hindered due to interaction between Me-18 and Me-19 as well as Me-20 and H-10 (Figure 5).[46]

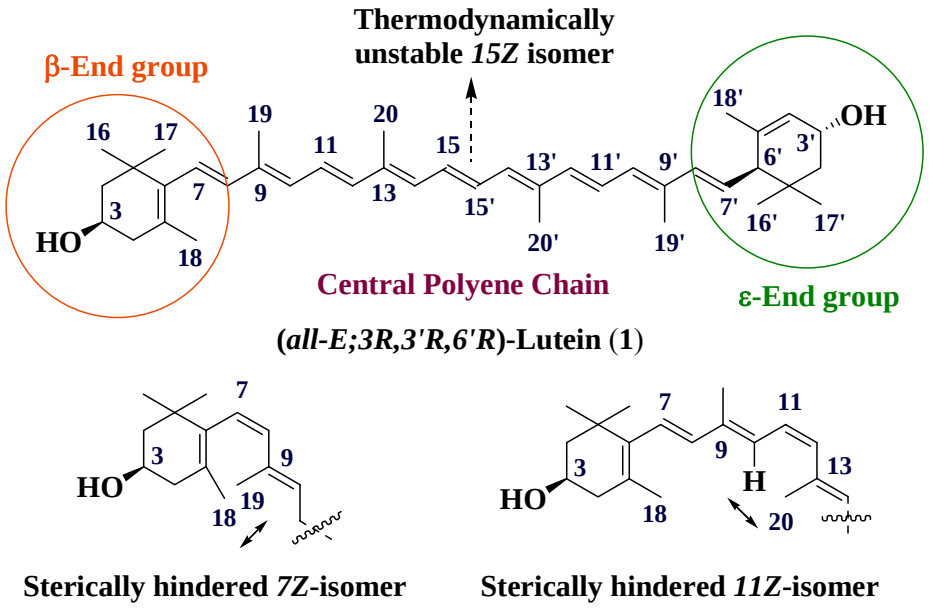
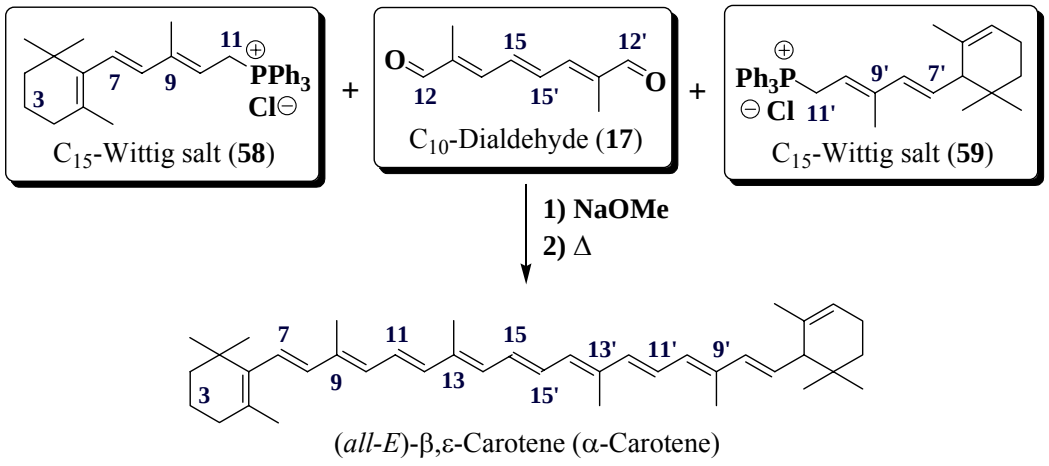


Figure 5. Structure of (*all-E;3R,3'R,6'R*)-lutein (**1**) and two of its sterically hindered stereoisomers.

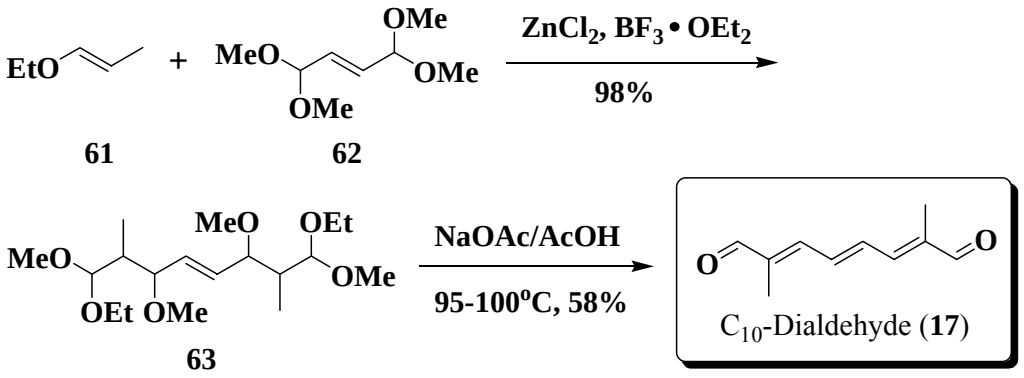
The *15Z*-isomers of carotenoids have been shown to be thermodynamically unstable and can be isomerized thermally or catalytically to their *all-E* isomers.[46] The occurrences of di-*Z*-stereoisomers of carotenoids in natural products are very rare. Therefore, the possible mono *Z*-isomers for lutein are *9Z*, *9′Z*, *13Z*, and *13′Z*.

The most common methodology for construction of carotenoid molecule is the Wittig reaction, employing C_{15}+C_{10}+C_{15} coupling strategy as shown in Scheme 10 for the synthesis of α-carotene.[38] This is because the C_{15}-end groups **58** and **59** and C_{10}-dialdehyde **17** can be synthesized with an *all-E* stereochemistry and the *11Z* isomer that may be formed in this coupling reaction can be readily isomerized to the more stable *all-E*-isomer.



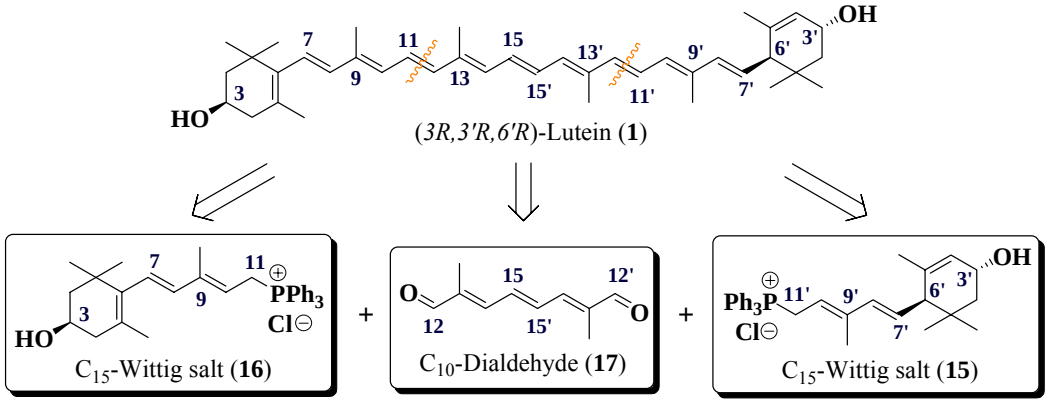
Scheme 10. C_{15}+C_{10}+C_{15} coupling strategy for the synthesis of α-carotene.[38]

The C_{10}-dialdehyde **17** has been prepared by many different routes. Among these, the synthesis of **17** by enol ether condensation is the most convenient and is used in industrial production (Scheme 11).[38] Therefore, the possible formation of *Z*-isomers of carotenoids according to this methodology is of little concern.



Scheme 11. One of the industrial routes to (*all-E*)-2,7-dimethylocta-2,4,6-triene- 1,8-dial (C_{10}-dialdehyde, **17**).[38]

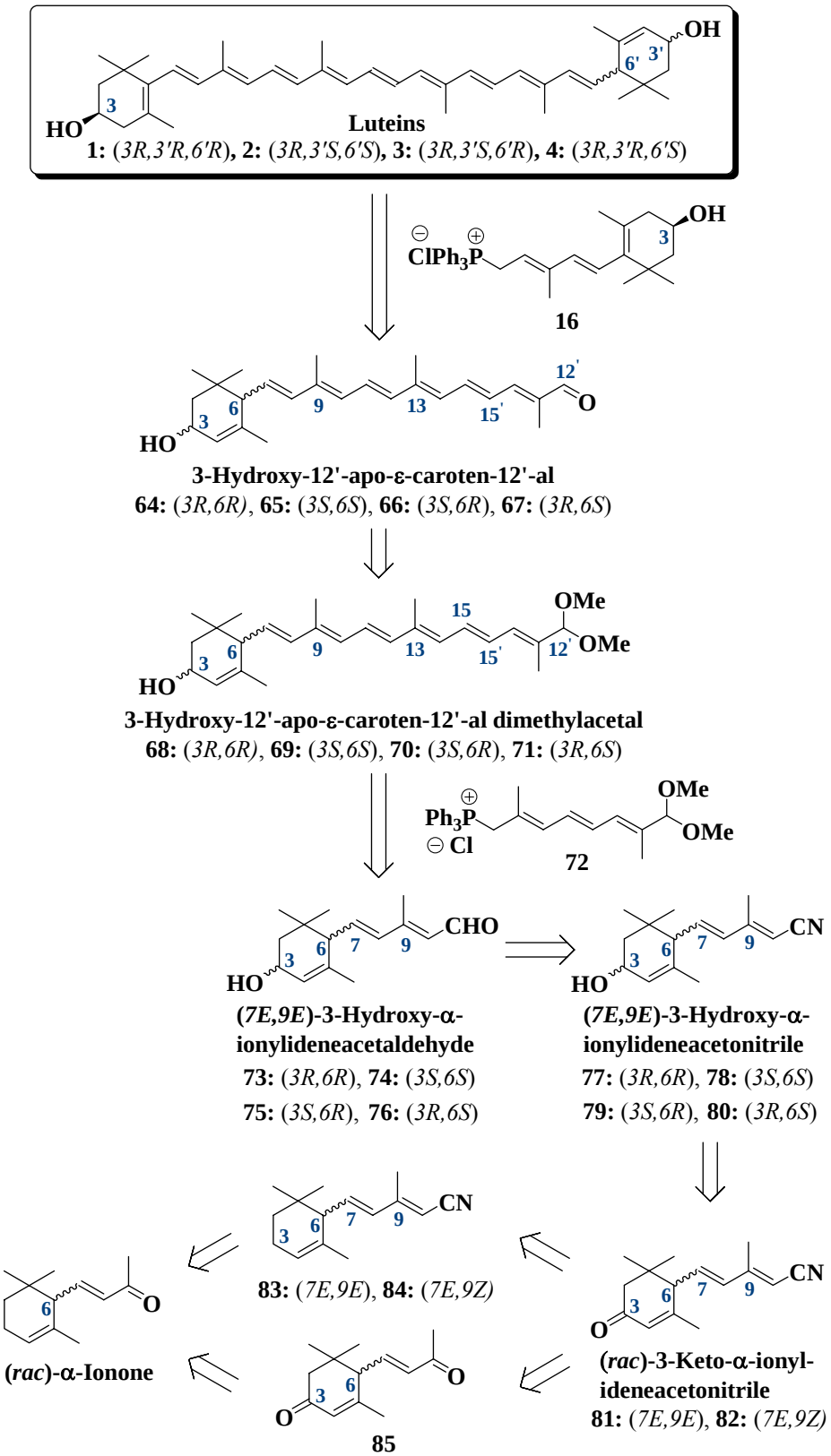
The synthesis of the (*7E,9E*)-C_{15}-end groups are more challenging due to the fact that the *9Z*-isomers of carotenoids are quite stable. For example, in the total synthesis of lutein **1** reported by Mayer and Rüttimann, the Wittig salts **15** and **16** were prepared as a mixture of (*9E*)- and (*9Z*)-isomers from which the *all-E* isomer was crystallized in low yield (Scheme 12).[37] Another drawback with the synthesis of lutein is the fact that the Wittig salt **15** which was used in the final coupling step, was prepared in a very poor yield due to the presence of the acid-sensitive allylic hydroxyl group.



Scheme 12. The final step of the total synthesis of (*3R,3′R,6′R*)-lutein (**1**) reported by Mayer and Rüttimann.[37]

Retrosynthetic Analysis of Luteins 1 – 4

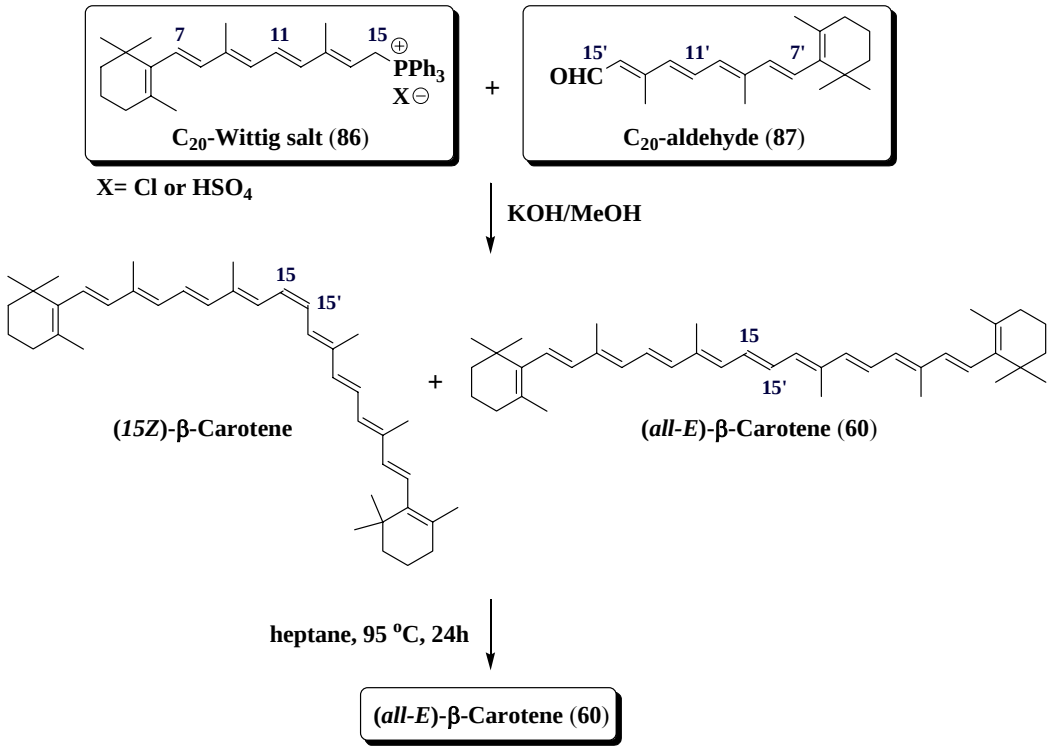
Although the C_{15}+C_{10}+C_{15} coupling strategy has been shown to be the method of choice for the synthesis of C_{40}-carotenoids, this approach has not been successfully applied to the synthesis of (*3R,3′R,6′R*)-lutein (**1**) by Mayer and Rüttimann.[37] Therefore, to accomplish the synthesis **1**, two retrosynthetic strategies were explored; the first strategy described in Scheme 13 is based on the C_{15}+C_{10}+C_{15} coupling strategy. In contrast to the reported synthesis of lutein, different building blocks were used, these were: 3-hydroxy-α-ionylideneacetaldehydes (C_{15}-aldehydes **73** – **76**), C_{10}-Wittig aldehydes **72** and C_{15}-Wittig salt **16**. It was anticipated that the final step of our synthesis could be readily accomplished by elongation of the optically pure C_{25}-hydroxyaldehydes **64** – **67** with the Wittig salt **16** that could be readily prepared according to the known processes.[44,45] In the latter part of this thesis, a novel route for the synthesis of Wittg salt **16** from (±)-α-ionone that has been developed in our laboratory will be described. We rationalized that the optically pure 3-hydroxy-12'-apo-ε-caroten-12'-al (C_{25}-hydroxyaldehydes **64** – **67**) could be prepared from deprotection of their corresponding dimethylacetals **68** – **71** under mild acidic conditions without epimerization of their allylic hydroxyl groups at C3. Each of these optically pure acetals could in turn be prepared from the reaction of protected C_{10}-Wittig salt **72** with the optically pure C_{15}-hydroxyaldehydes **73** – **76** with the required stereochemistry at C3 and C6. The protected C_{10}-Wittig salt **72** was readily accessible according to published methods.[47,48]



Scheme 13. Retrosynthetic strategy 1 for total synthesis of luteins **1** – **4** using C_{15}+C_{10}+C_{15} building blocks.

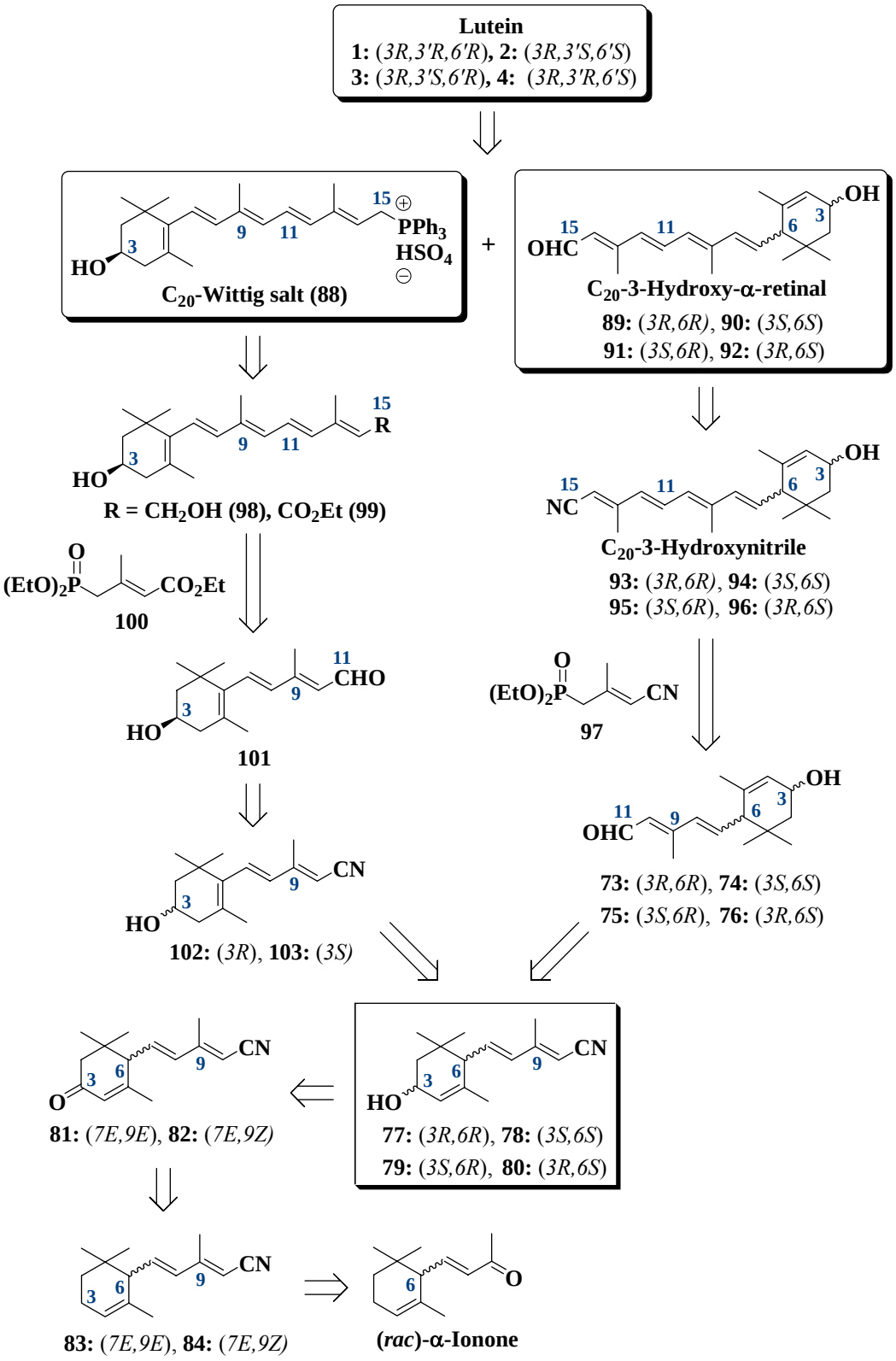
The application of this Wittig salt **72** in the synthesis of unsymmetrical carotenoids with sensitive end-groups has been well documented in the literature.[48-51] However, this building block has not been employed in the synthesis of lutein or its precursors. The C_{15}-hydroxynitriles **77** – **80** as a racemic mixture or with the appropriate stereochemistry at C3 and C6 could serve as the precursors to C_{15}-hydroxyaldehydes **73** – **76**. (*7E,9E*)-3-Keto-α-ionylideneacetonitrile (**81**) and its (*7E,9Z*)-isomer (**82**), prepared from allylic oxidation of nitriles **83** and **84**, could be transformed into C_{15}-hydroxynitriles **77** – **80**. We envisioned that either the diastereomeric hydroxynitriles or hydroxyaldehydes could be first separated from their respective racemic mixtures into two pairs of enantiomers by chromatography and each pair could then be resolved by enzyme-mediated acylation. However the (*7E,9E*)-isomer (**81**) would be preferable since this would avoid the difficulties associated with the separation of optically active 9*E*/9*Z*-isomers throughout our entire synthetic strategy. The commercially available and inexpensive (±)-α-ionone was selected as the starting material for the synthesis of ketonitriles **81** and **82**. Ketonitriles **81** and **82** have been previously synthesized as a mixture of *E/Z*-isomers from (±)-α-ionone via (±)-3-keto-α-ionone (**85**) by Wadsworth-Horner-Emmons (WHE) olefination.[52] Therefore, we had to develop a methodology that could provide **83** as a single isomer and transform this nitrile into **81** without stereoisomerization. Other challenges with our synthetic approach were separation of C_{15}-hydroxyaldehydes **73** – **76** or their precursors, C_{15}-hydroxyanitriles **77** – **80**, in high optical purity and maintaining their stereochemical integrity throughout the synthesis of luteins **1** – **4**.

Another possible route for the synthesis of carotenoids is by employing a C_{20}+C_{20} coupling strategy. As mentioned earlier, any amount of *15Z*-isomer that is formed as a result of this coupling can be transformed into the thermodynamically more stable *all-E*-isomer, simply by refluxing in an organic solvent at high temperatures. An example of this is the total synthesis of β-carotene (**60**) by Wittig reaction of retinyltriphenylphosphonium salt **86** with retinal **87** as shown in Scheme 14.[53-55]



Scheme 14. Synthesis of (*all-E*)-β-carotene (**60**) by C_{20}+C_{20} coupling strategy.[53-55]

Thus, our second strategy focused on the reaction of C_{20}-Wittig salt **88** and C_{20}-3-hydroxy-α-retinals (C_{20}-hydroxyaldehydes **89** – **92**) in a final step to arrive at luteins **1** – **4** (Scheme 15).



Scheme 15. Retrosynthetic strategy 2 for total synthesis of luteins **1** – **4** using C_{20}+C_{20} building blocks.

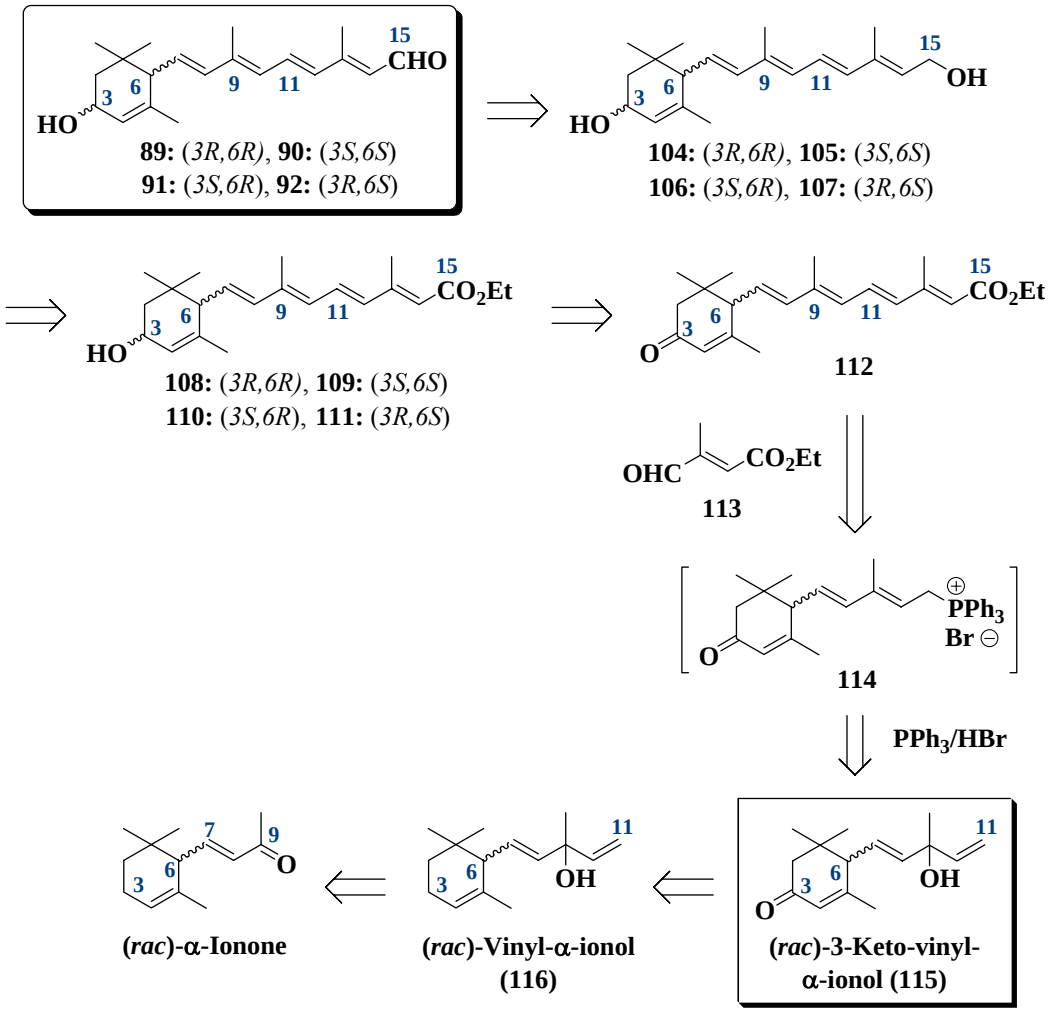
The selection of the C_{20}-Wittig salt **88** and the C_{20}-hydroxyaldehydes **89** – **92** was based on the position of the hydroxyl groups in the end-groups of these precursors. The C_{20}-hydroxyaldehydes **89** – **92** have allylic hydroxyl groups that are sensitive to acids. Therefore, it was anticipated that the preparation of the corresponding Wittig salts from these end-group would be problematic and accompanied by elimination of water. On the contrary, the preparation of the C_{20}-Wittig salt **88** with a β-end-group in which the hydroxy group is not allylic was not expected to present this problem. As described earlier in Scheme 14, C_{20}-Wittig salt **86**, which has been used in the synthesis of β-carotene, has been successfully prepared. However, it was unclear to us whether the presence of the non-allylic hydroxyl group could interfere with the preparation of this Wittig salt. It was envisioned that both of these C_{20}-end groups **88** and **89** – **92**, could be prepared from a non-chiral and commercially available common precursor such as (±)-α-ionone by convergent synthesis.

As illustrated in Scheme 15, racemic mixture or optically pure hydroxynitriles **77** – **80** could serve as the key intermediates for synthesis of both C_{20}-Wittig salt **88** and C_{20}-hydroxyaldehydes **89** – **92**. These C_{20}-hydroxyaldehydes **89** – **92** could be prepared from the reduction of their corresponding C_{20}-hydroxynitriles **93** – **96**. These nitriles **93** – **96** would be accessible from WHE olefination of C_{15}-hydroxyaldehydes **73** – **76** with the C_5-synthon, 4-(diethylphosphono)-3-methyl- 2-butenenitrile (**97**). As described earlier (see Scheme 13), (±)-α-ionone could serve as the starting material for the synthesis of C_{15}-hydroxyaldehydes **73** – **76**. The separation of the optically pure hydroxyaldehydes **73** – **76** or their nitrile precursors **77** – **80** could be

accomplished by combination of chromatography and enzyme-mediated acylation as described earlier.

We planned to prepare the C_{20}-Wittig salt **88** from 3-hydroxy-retinol (**98**) according to known procedures. This precursor could be readily obtained from reduction of hydroxyester **99**. The optical resolution of nitriles **102** and **103** would be achieved and nitrile **102** coule serve as the precursor to **101**. We rationalized that since the H-6 hydrogen in hydroxynitriles **77** – **80** are fairly acidic, the optically pure or racemic mixture of these nitriles could readily undergo base-catalyzed isomerization to nitriles **102** and **103**.

We could also arrive at the C_{20}-hydroxyaldehydes **89** – **92** from their corresponding diols **104** – **107** (Scheme 16). These diols would be readily accessible from ketoester **112** via hydroxyesters **108** – **111**. The Wittig reaction of Wittig salt **114** with ethyl-3-formyl-2-butenoate **113** would provide the ketoester **112**. The Wittig salt **114** has been previously prepared from (±)-α-ionone via 3-keto-vinyl-α-ionol (**115**).[37] According to this route, enzymatic acylation of hydroxyesters **108** – **111** in combination with chromatography could be employed to separate these esters and provide optically pure aldehydes **89** – **92**.



Scheme 16. Alternative route to the synthesis C_{20}-3-hydroxy-α-retinals (C_{20}-hydroxyaldehydes, **89** – **92**).

Synthesis of Lutein and its Stereoisomers According to Strategy 1 Employing C_{15}+C_{10}+C_{15} Coupling Reaction

The initial challenge for the synthesis of luteins **1** – **4** was to develop a methodology that could provide (*7E,9E*)-3-keto-α-ionylideneacetonitrile (**81**) as single isomer. This is because when this (*7E,9E*)-isomer **81** is reduced in the

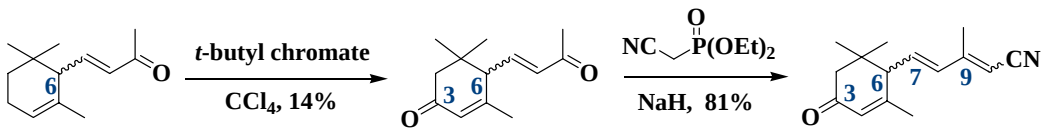
following step, a new stereogenic center at C3 is generated that results in the formation of four stereoisomeric (±)-hydroxynitriles **77** – **80** (Figure 6).

81: (*7E,9E*)
82: (*7E,9Z*)

77 – 80
(*7E,9E*)-isomers + (*7E,9Z*)-isomers

Figure 6. Structures of ketonitriles **81** and **82** and their reduction products.

Consequently, the reduction of a mixture of (*7E,9E*)–**81** and (*7E,9Z*)–**82** could afford as many as 8 stereoisomeric hydroxynitriles which would be difficult to separate in high optically purity. Therefore, the initial goal was to explore the possible routes by which (±)-α-ionone could be transformed mainly into (*7E,9E*)-ketonitrile **81**. For this purpose, we first investigated the reported transformation of (±)-α-ionone to ketonitriles **81** and **82** according to the method of Imai et al. (Scheme 17).[52]



(*rac*)-α-ionone

3-Keto-α-ionone (**85**)

3-Keto-α-ionyl-ideneacetonitrile
81: (*7E,9E*) / **82:** (*7E,9Z*)

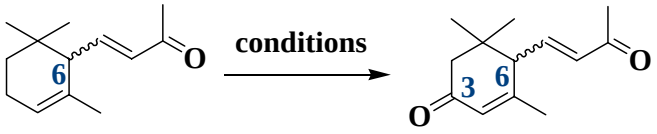
Scheme 17. Synthesis of 3-ketonitrile **81** and **82** by Imai et al.[52]

These authors obtained ketonitriles **81** and **82** via 3-keto-α-ionone (**85**) in 81% yield as an oil; however the isomeric ratio of **81/82** was not reported. Therefore, we investigated this reaction to determine the isomeric ratio of these ketonitriles.

There are several published methodologies for oxidation of (±)-α-ionone to 3-keto-α-ionone (**85**). The first was reported by Prelog and Osgan in 1952 that involved using *tert*-butyl chromate to oxidize(±)-α-ionone to **85** in 14% isolated yield.[56]

Widmer et al. employed $Ac_2Co.4H_2O/NH_4Br/O_2$ to improve the yield of this reaction to 31%.[44] More recently, another procedure for allylic oxidation of ionone-like dienes with TBHP catalyzed by $CaCl_2$ and $MgCl_2.6H_2O$ at 60°C has also been reported that can afford **85** in 67% isolated yield.[57] Imai et al. used the method of Prelog and Osgan to prepare **85**.[52] However, since this method only gave 14% yield of ketoionone, we first investigated this reaction employing 3 different oxidizing reagents and the results are shown in Table 1.

Table 1. Allylic oxidation of (±)-3-keto-α-ionone (**85**).



(*rac*)-α-ionone **3-Keto-α-ionone (85)**

Entry	Conditions	Isolated Yield (%)
1	Pd/C, 5.5 M TBHP in decane, K_2CO_3, CH_2Cl_2, 0˚C to R.T., 36 h	53%
2	$Rh_2(cap)_4$[a], 5.5 M TBHP in decane, K_2CO_3, CH_2Cl_2, R.T., 22 h	63%
3	Household bleach, 70% aq. TBHP, K_2CO_3, EtOAc, -5 to 0˚C, 5 h	64%

[a] $Rh_2(cap)_4$ = Dirhodium (II) caprolactamate

We first tried the oxidation method of α-ionone similar to the Pd(II)-mediated allylic oxidation of cyclic alkenes developed by Yu and Corey (Table 1, entry 1).[58] Under this condition, (±)-α-ionone was oxidized to 3-keto-α-ionone (**85**) in 53% isolated yield. We were also able to prepare **85** in 63% yields by catalytic oxidation

with dirhodium(II) caprolactamate that has been recently developed by Catino et al. for allylic oxidation of olefins (Table 1, entry 2).[59] While both of these methodologies provided **85** in moderate yields, their main drawback was the use of dry and concentrated TBHP (5.5M in decane) that could be difficult to handle on a large industrial scale.

Recently, a water-based oxidation system, using household laundry bleach and aqueous TBHP (70% in water), has been shown to convert steroidal olefins to α,β-enones in an economical and environmentally friendly manner.[60] Employing this bleach oxidation, we prepared crystalline **85** from (±)-α-ionone in 64% isolated yield (Table 1, entry 3). The proposed mechanisms of these allylic oxidation reactions with Pd/C/TBHP and bleach/TBHP will be discussed later.

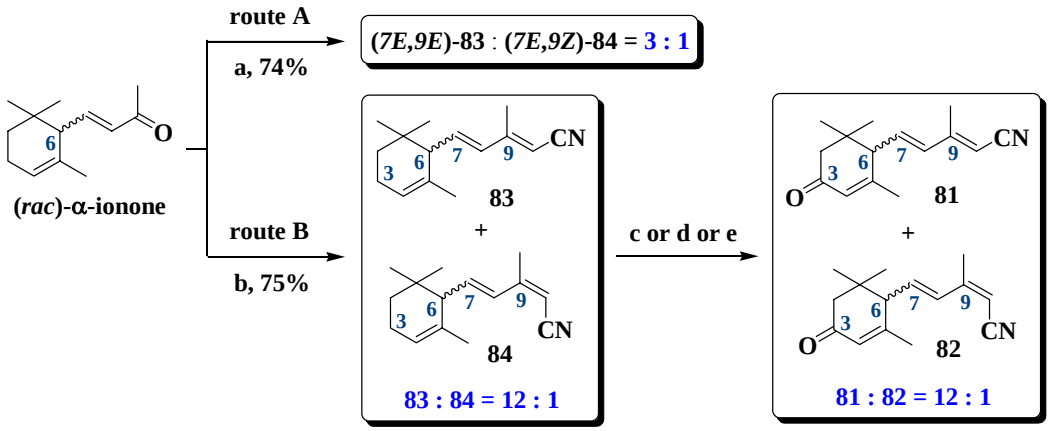
Next, we investigated the WHE reaction of (±)-3-keto-α-ionone (**85**) with diethyl cyanomethylphosphonate in THF according to the method of Imai et al.[52] and obtained a mixture of **81**: **82** = 3:1 in 81% yield after purification by chromatography (Scheme 17). The isomeric ketonitriles **81** and **82** were subjected to low temperature crystallization (–15^{o}C) in ethanol that afforded **81** as white crystals in 40% isolated yield.

To increase the *E/Z* ratio, we attempted the WHE reaction of **85** with diisopropyl cyanomethylphosphonate because this bulky phosphonate has been used in the WHE reaction with β-ionone to significantly increase the *E/Z* ratio of the product.[60] In our case, this phosphonate only marginally improved the *E/Z* ratio to 4/1.

Due to the fact that considerable amounts of (*7E,9Z*)-isomer of ketonitriles **82** was present in the product, the crystallization of the (*7E,9E*)-isomer **81** could only be

accomplished after 2 weeks at –15°C. In addition, when this reaction was scaled up, the yield of isomeric mixture of **81** and **82** after chromatography dropped to 40%.

To overcome these problems, we decided to first convert α-ionone to α-ionylideneacetonitriles (**83** and **84**) and then performed the allylic oxidation to obtain ketonitriles **81** and **82** (Scheme 18). α-Ionone was transformed to nitriles **83** and **84** under two different sets of reaction conditions.

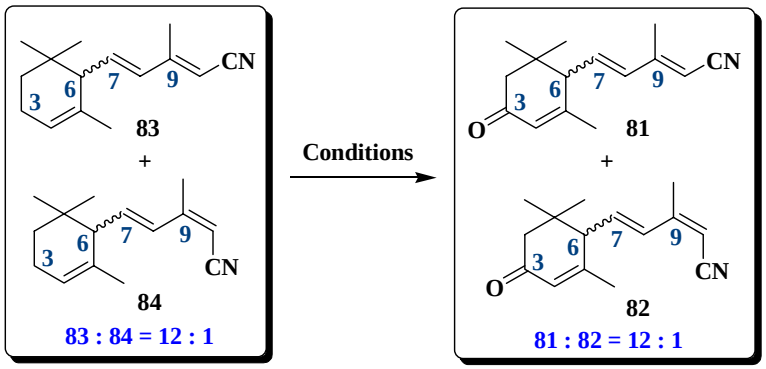


Scheme 18. Synthesis of (*7E,9E*)-3-keto-α-ionylideneacetonitriles (**81**) from (±)-α-ionone. **a)** $(EtO)_2P(O)CH_2CN$ or $(i\text{-}PrO)_2P(O)CH_2CN$/NaH, TBME or THF or NaOMe/MeOH; **b)** $CH_2(CN)CO_2H$, cyclohexylamine, 80-85°C, 3.5 h. **c**) Pd/C, TBHP, K_2CO_3; **d**) $Rh_2(cap)_4$, TBHP, K_2CO_3; **e**) Household bleach (5.25% NaOCl), 70% TBHP, K_2CO_3.

The WHE reaction of (±)-α-ionone with diethyl cyanomethylphosphonate in TBME using NaH as base gave nitriles **83** : **84** = 3 : 1 in 74% isolated yield after distillation (Scheme 18, route A). Further, the use of diisopropyl cyanomethylphosphonate did not improve the *E/Z* ratio.[61] Therefore, in an attempt to increase the *E/Z* ratio of nitriles **83/84**, we examined the Knoevenagel reaction of (±)-α-ionone with cyanoacetic acid (Scheme 18, route B). In 1944, Young et al. prepared α-ionylideneacetonitrile from condensation of α-ionone with cyanoacetic acid using a

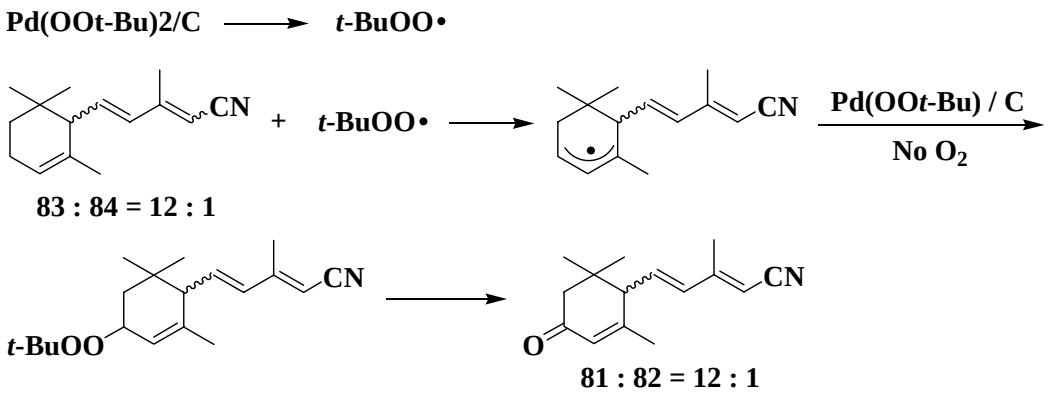
mixture of acetamide and ammonium acetate as catalyst.[62] However, due to the old nature of this publication and lack of sophisticated analytical methods at that time, the *E*/*Z*-ratio of these nitriles could not be determined. More recently, Knoevenagel condensation of β-ionone with cyanoacetic acid in boiling pyridine and catalytic amounts of piperidinium acetate has been shown to afford β-ionylideneacetonitrile in high yield, predominantly as the (*7E,9E*)-isomer.[63] When we applied these conditions to the condensation of (±)-α-ionone with cyanoacetic acid, no reaction was observed. After examining this reaction with a number of organic amines, we discovered that cyclohexylamine could promote this reaction under mild conditions to give a 75% yield of **83** : **84** = 12 : 1 as a colorless oil after distillation (Scheme 18, route B). With these nitriles in hand, we explored the oxidation reaction of nitriles **83** and **84** with three oxidizing reagents (Table 2).

Table 2. Allylic oxidation of nitrile **83** and **84** under three different conditions.



Entry	Conditions	Isolated Yield %
1	Pd/C, 5.5M TBHP in decane, K_2CO_3, CH_2Cl_2, 0˚C to R.T., 3 days	53%
2	$Rh_2(cap)_4$, 5.5M TBHP in decane, K_2CO_3, CH_2Cl_2, R.T., 24 h	N.R.
3	Household bleach (5.25% NaOCl), 70% TBHP in H_2O, K_2CO_3, CH_3CN, -2 to 5˚C, 12 h	57%

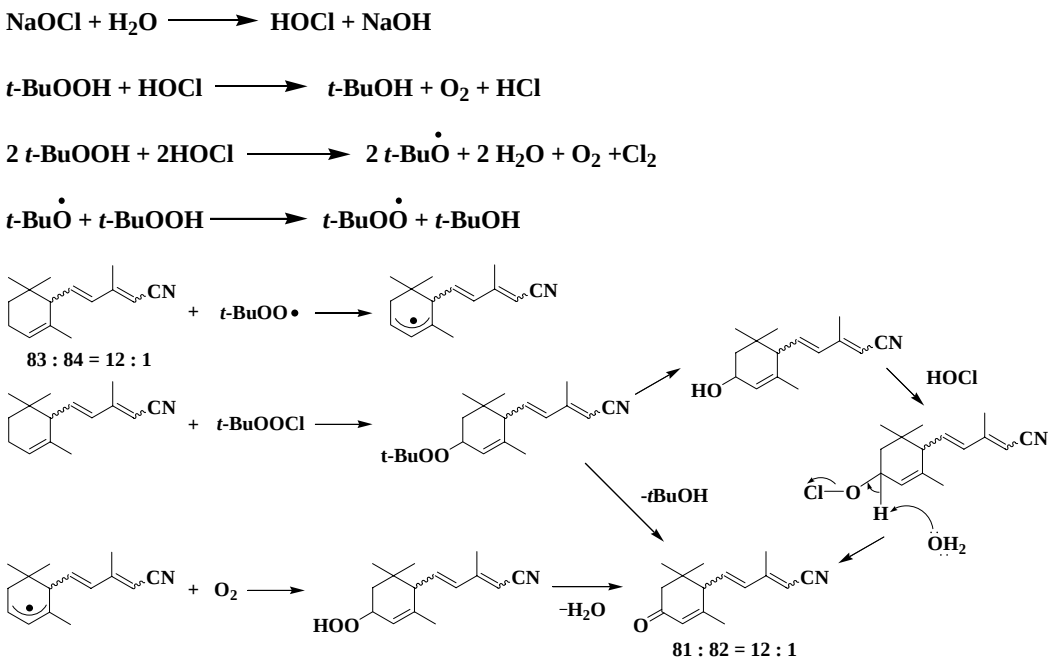
Ketonitriles **81** and **82** were prepared in 12 to 1 ratio in 53% yield by palladium-mediated oxidation of nitriles **83** and **84** with TBHP in CH_2Cl_2 at 0°C by applying the method developed by Yu and Corey as described before (Table 2, entry 1).[58] The mechanism of this palladium-catalyzed allylic oxidation modified for our substrate is shown in Scheme 19. According to the authors, this oxidation involves the formation of allylic *tert*-butylperoxy ether intermediate which is further oxidized to form an α,β-enone.



Scheme 19. The mechanism of Pd-mediated oxidation of nitriles **83** and **84** using method of Yu and Corey.[58]

The catalytic oxidation of nitriles **83**/**84** with $Rh_2(cap)_4$/TBHP according to the method of Catino et al. was unsuccessful (Table 2, entry 2).[59] Fortunately, the bleach oxidation of a mixture of nitriles **83** and **84** with *tert*-BuOOH (TBHP, 70% in water), household bleach (5.25% NaOCl), and catalytic amounts of K_2CO_3 in CH_3CN at -5 to 0°C afforded **81** : **82** = 12 : 1 (Table 2, entry 3).[60] After purification by chromatography, a mixture of these ketonitriles was obtained in 57% yield. This

mixture was crystallized from ethanol at -15 °C to give the **81** as white crystals free from **82** in 37% isolated yield. The proposed mechanism of bleach oxidation for nitriles **83/84** is shown in Scheme 20 and involves generation of *tert*-butylperoxide radical which initiates the formation of the allylic radical of the substrate. The reaction of hypochlorite with TBHP is highly exothermic and generates considerable amount of oxygen in situ and as a result, the temperature of this reaction should be preferably kept below 5°C. The authors have demonstrated that at higher temperature, the yield of this oxidation is substantially decreased.



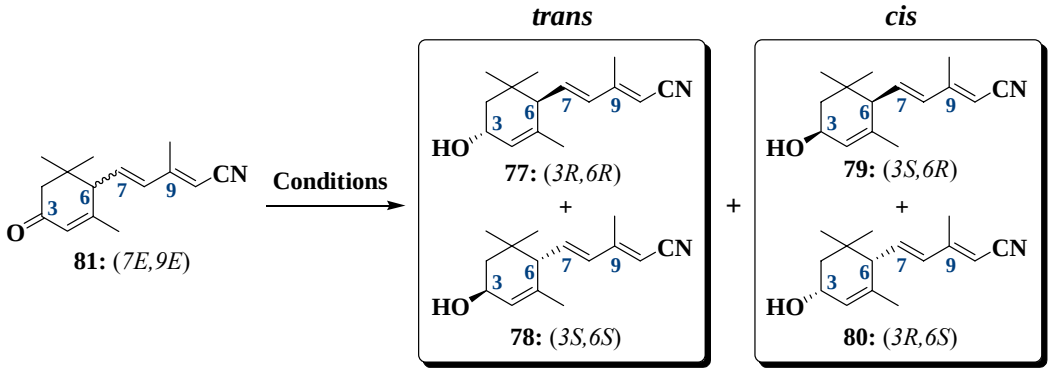
Scheme 20. The mechanism of bleach oxidation of nitriles **83** and **84**.[60]

These oxidation reactions clearly revealed that upon oxidation of nitriles **83/84** to **81/82**, the *E*/*Z* isomeric ratio remained unchanged. It should be noted that the direct oxidation of nitriles **83/84** to **81/82** has not been previously reported. While the

overall yield of ketonitriles **81** and **82** by routes A and B discussed above were comparable, route B proved to be easier to scale up and due to the high *E*-stereoselectivity of the Knoevenagel condensation, **81** could be crystallized from the isomeric mixture more expeditiously.

The reduction of (*7E,9E*)-ketonitrile **81** that gave four stereoisomeric hydroxynitriles **77** – **80** was explored with a number of reagents and the results are shown in Table 3. Because (*3R,6R*)-hydroxynitrile **77** with a *trans* relationship between the OH at C3 and C6-dienenitrile side chain is the precursor of the naturally occurring (*3R,3'R,6'R*)-lutein (**1**), it was desirable to increase the composition of the (*trans*)-hydroxynitriles **77** and **78** relative to the (*cis*)-hydroxynitriles **79** and **80** in the reduction products.

Table 3. Reduction of ketonitrile **81** to hydroxynitriles **77** – **80** with various reagents.

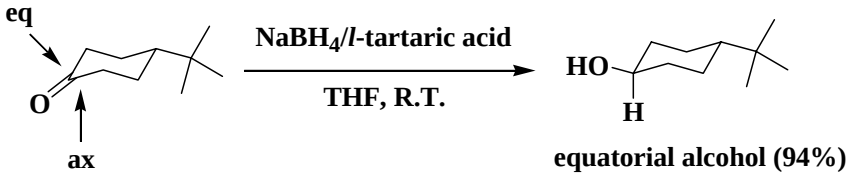


Entry	Conditions	(**77**/**78**):(**79**/**80**) (*trans:cis*)[a]	Conversion (%)[b]
1	$NaBH_4$, EtOH, H_2O, 0°C to R.T., 24 h	1.0 : 1.0	97 [c]
2	Triisobutylaluminum (TIBA), Toluene, -40°C to R.T., 1 h	2.0 : 3.0	95
3	$NaBH_4$/*d*-Tartaric acid (3/1), EtOH, -10 to -15°C, 2 h	3.0 : 1.0	94
4	$NaBH_4$/*l*-Tartaric acid (3/1), EtOH, - 10 to -15°C, 2 h	3.0 : 1.0	94
5	$NaBH_4$/Dibenzoyl-*d*-tartaric acid (3/1), EtOH, - 10 to -15°C, 2 h	3.0 : 1.0	96
6	$NaBH_4$/*dl*-Tartaric acid (3/1), EtOH, -10 to -15°C, 2 h	3.0 : 1.0	94
7	Sodium bis(2-methoxyethoxy)aluminum hydride, $NaAlH_2(OCH_2CH_2OMe)_2$ = (Red-Al™), TBME, -5 to 0 °C, 1 h	1.3 : 1.0	95
8	Lithium tri-*sec*-butylborohydride, $LiB[CHMeCH_2CH_3]_3H$ (L-Selectride™), TBME, -30°C, 0.5 h	1.2 : 1.0	93
9	Sodium tri-*sec*-butylborohydride, $NaB[CHMeCH_2CH_3]_3H$ (N-Selectride™), TBME, -30°C, 0.5 h	2.5 : 1.0	92
10	Potassium trisiamylborohydride, $KB[CHMeCHMe_2]_3H$ (KS-Selectride™), TBME, -30 to 0°C, 2 h	2.2 : 1.0	91
11	**Potassium tri-*sec*-butylborohydride, $KB[CHMeCH_2CH_3]_3H$ (K-Selectride™), TBME, -30°C, 0.5 h**	**6.0 : 1.0**	96[c]
12	**BH_3/(*R*)-2-methyl-CBS-oxazaborolidine, TBME, 0°C, 1.5 h**	**1.0 : 6.0**	90[c]
13	BH_3/ (*S*)-2-methyl-CBS-oxazaborolidine, TBME, 0°C, 1.5 h	1.0 : 3.0	93

[a] Ratios were determined by HPLC on a silica-based nitrile bonded column and a chiral column (Eluent A and Eluent C, see Appendix I). [b] Conversion to product was determined by HPLC (Eluent A). [c] Isolated yield after chromatography.

Reduction of the ketonitrile **81** with $NaBH_4$ was sluggish and gave *trans*-hydroxynitriles (**77+78**) and *cis*-hydroxynitriles (**79+80**) in nearly equal amounts and was not stereoselective (Table 3, entry 1). The ratio of **77+78** to **79+80** was determined by HPLC analysis of the products employing a silica-based nitrile bonded column that readily separated the *trans*-hydroxynitriles (**77**+**78**) from the *cis*-hydroxynitriles (**79**+**80**) (Eluent A, Appendix I). While the reduction with TIBA was quite efficient even at low temperature (-40°C), the relative composition of *trans*-hydroxynitriles (**77**+**78**) to *cis*-hydroxynitriles (**79**+**80**) was not dramatically affected (Table 3, entry 2).

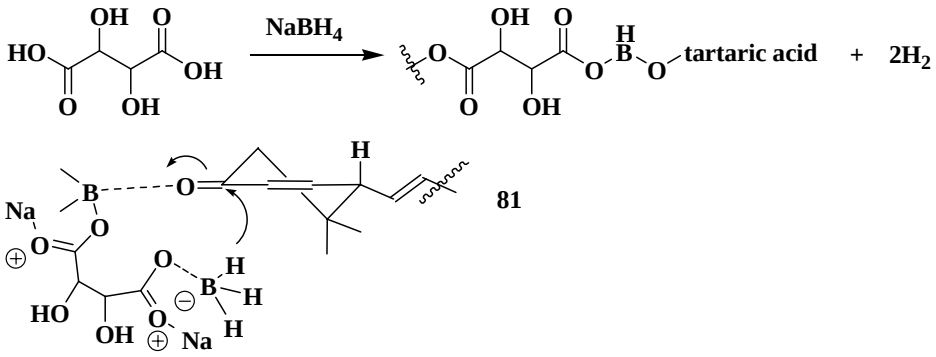
There are several literature report on the use of the combination of $NaBH_4$ and chiral tartaric acid and its derivatives in the reduction of ketones but none of these examples involve the reduction of cyclic α,β-enones.[64-67] For example, *l*-tartaric acid has been successfully applied to the reduction of cyclic ketones affording equatorial alcohols as the major product and the axial alcohol as the minor product.[67] It has been proposed that the resulting complex from $NaBH_4$ and carboxylic acid, acyloxyborohydride, acts as an axial hydride donor in order to produce the equatorial alcohol (Scheme 21).[67-68]



Scheme 21. Reduction of 4-*tert*-butylcyclohexanone with $NaBH_4$/*l*-tartaric acid.[67]

When we employed a combination of *d*-tartaric acid and $NaBH_4$, we were pleasantly surprised to obtain *trans*-hydroxynitriles (**77**+**78**) : *cis*-hydroxynitriles (**79**+**80**) = 3 : 1. Interestingly we obtained exactly the same product ratio with *l*-tartaric acid and $NaBH_4$; this finding prompted us to investigate the reduction of ketonitriles **81** with the inexpensive *dl*-tartaric acid.

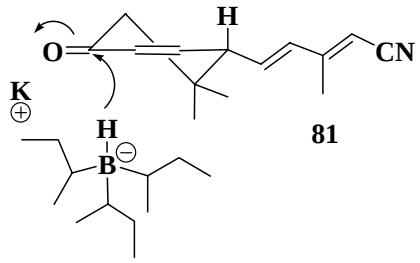
Ironically, this reagent was found to be as efficient as enantiomerically pure *d*- or *l*-tartaric acid (Table 3, entries 3-6). Consequently in our case, the stereochemistry of the reducing agent does not seem to influence the stereoselectivity of this reduction. Therefore, $NaBH_4$/tartaric acid complex may add the hydride preferably to the pseudoaxial position of the nitrile **81** to afford a greater proportion of *trans*-hydroxynitrile relative to the *cis*-nitriles (Scheme 22).



Scheme 22. Proposed mechanism for the reduction of **81** using $NaBH_4$/tartaric acid.

Red-Al™ [sodium bis(2-methoxyethoxy)aluminum hydride] is a common and convenient reducing agent that is highly efficient in the reduction of ketones. However, as expected, the reduction of **81** with Red-Al™ did not show a significant preference for the formation of *trans*-hydroxynitriles (**77+78**) (Table 3, entry 7).

Bulky trialkylborohydrides (Selectrides™) have been shown to be promising reagents for stereoselective reduction of rigid cyclic enones and yield the thermodynamically more stable pseudoequatorial alcohol as the major product.[69-72] Encouraged by these literature reports, we surveyed four different Selectrides, these were: L-Selectride™, N-Selectride™, K-Selectride™ and KS-Selectride™. The reduction of **81** with lithium tri-*sec*-butylborohydride (L-Selectride™) did not show a significant preference for the formation of *trans*-hydroxynitriles (**77+78**) (Table 3, entry 8). When sodium tri-*sec*-butylborohydride (N-Selectride™) or potassium trisiamylborohydride (KS-Selectride™) were employed as the reducing agents, the relative composition of *trans*-hydroxynitriles (**77**+**78**) to *cis*-hydroxynitriles (**79**+**80**) was 2.5 : 1 and 2.2 : 1, respectively (Table 3, entries 9 and 10). Surprisingly, the best stereoselectivity was achieved when **81** was reduced with potassium tri-*sec*-butylborohydride (K-Selectride™) which gave *trans*-hydroxynitriles as the major product and the *cis*-hydroxynitriles as the minor product (**77+78/79+80** = 6/1) (Table 3, entry 11). Since N-Selectride™, L-Selectride™, and K-Selectride™ bear the same tri-alkyl groups; our observed stereoselectivity with the latter Selectride is probably due to the size of the metal ion (Scheme 23). The large size of potassium relative to lithium and sodium may be responsible for the formation of the borohydride complex with ketone **81** from the less sterically hindered side resulting in pseudoaxial hydride addition.



Scheme 23. Formation of the complex between ketone **81** and K-Selectride™.

Contrary to the results obtained with K-Selectride™, the reduction of ketonitrile **81** with BH_3/(*R*)-2-methyl-CBS-oxazaborolidine according to the method of Corey et al.[73-74] gave *trans*-hydroxynitriles (**77**+**78**) as the minor product and the *cis*-hydroxynitriles (**79**+**80**) as the major product in 1:6 ratio (Table 3, entry 12). When BH_3/(*S*)-2-methyl-CBS-oxazaborolidine was used as the reducing agent, the *cis*-hydroxynitriles (**79**+**80**) were still obtained as the major products but the stereoselectivity was not as high as that obtained with the *R*-isomer of CBS-oxazaborolidine (Table 3, entry 13). These contradictory results are not clearly understood at this time.

To summarize the outcome of the reduction reactions described in Table 3, we were able to develop methods for the reduction of **81** to *trans*-hydroxynitriles (**77**+**78**) and *cis*-hydroxynitriles (**79**+**80**) in ratios ranging from 6:1 and 1:6.

Enzyme-Mediated Separation of Hydroxynitriles 77 – 80

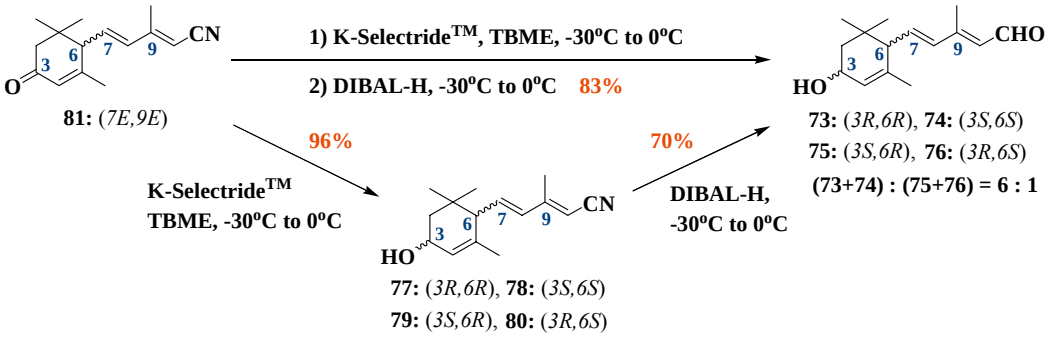
As described in the retrosynthetic analysis described in Scheme 13, we anticipated that *trans*-hydroxynitriles **77+78** could first be separated from their diastereomers, *cis*-hydroxynitriles **79+80** by column chromatography; subsequently

each pair of enantiomer would be subjected to enzyme-mediated acylation to resolve these nitriles. This idea was inspired from a literature report that has clearly demonstrated that 3′-epilutein [(*3R,3′S,6′R*)-lutein (**3**)] can be separated from (*3R,3′R,6′R*)-lutein (**1**) by enzyme-mediated acylation with lipase AK (*Pseudomonas fluorescens*) in 90% *de*.[40] It should be noted that this literature report is the only example for separation of diastereomeric carotenoids by enzymatic acylation and to our knowledge, there is no literature report on separation of enantiomeric carotenoids or their C_{15}-precursors. However, there are numerous literature reports on resolution of cyclic alcohols by enzyme mediated acylation.[40,75,76] The separation of *trans*-hydroxynitriles **77**+**78** from *cis*-hydroxynitriles **79+80** by column chromatography proved to be challenging and this could only be accomplished by subjecting these nitriles to several column chromatographic separations. In addition, attempts to resolve the (±)-hydroxynitriles **77+78** or **79+80** by enzyme-mediated acylation with lipase AK (*Pseudomonas fluorescens*) or lipase PS (*Pseudomonas cepacia*) resulted in poor *ee* for these nitriles. Discouraged by these findings, other enzymes were not investigated and the separation of these hydroxynitriles was postponed until after reduction to their corresponding hydroxyaldehydes **73** – **76**.

One-Pot Reduction of Ketonitrile 81 to Hydroxyaldehydes 73 – 76 *via* Hydroxynitriles 77 – 80

The reduction of hydroxynitriles **77** – **80** with DIBAL-H in dichloromethane afforded a mixture of the four hydroxyaldehydes **73** – **76** in 70% isolated yield after chromatography (Scheme 24). In the following step, a mixture of *trans*-C_{15}-

hydroxyaldehydes **73**+**74** was readily separated from a mixture *cis*-C_{15}-hydroxyaldehydes **75**+**76** by column chromatography. Hydroxyaldehydes **73** – **76** could also be prepared in an efficient one-pot reaction using K-Selectride™ followed by reduction with DIBAL-H to yield **73+74** and **75+76** in the ratio of 6:1 in 83% isolated yield (Scheme 24).



Scheme 24. Reduction of ketonitrile **81** to aldehydes **73** – **76** via nitriles **77** – **80**.

The ratio of *trans*-C_{15}-hydroxyaldehydes **73+74** to *cis*-C_{15}-hydroxyaldehydes **75+76** was determined by HPLC analysis of the products employing a silica-based nitrile bonded column (Eluent A) that separated the **73**+**74** from **75**+**76** efficiently (Figure 7).

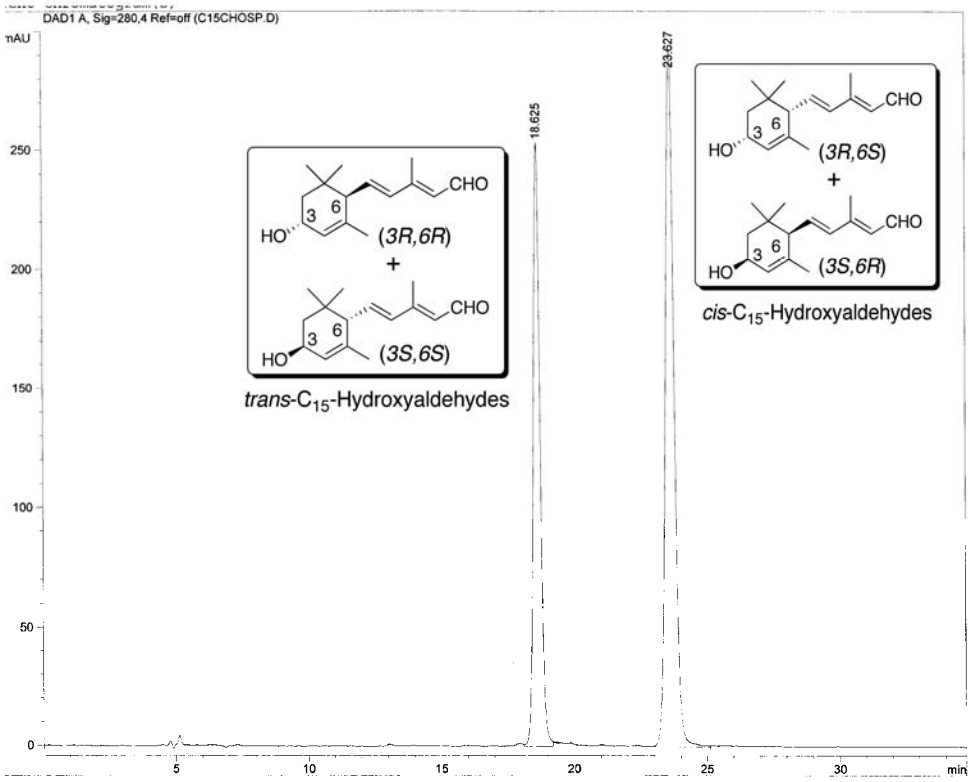
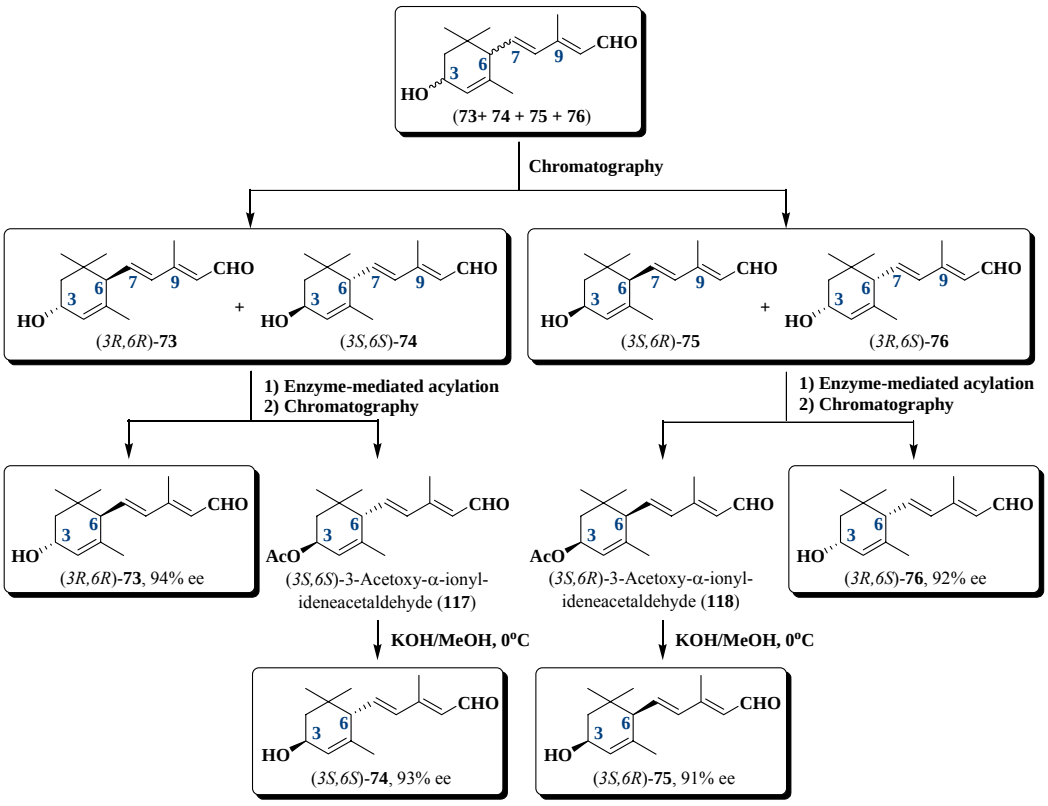


Figure 7. The HPLC profile of a racemic mixture of hydroxyaldehydes **73** – **76**. HPLC conditions (Eluent A) is described in Appendix I.

Enzyme-Mediated Separation of Hydroxyaldehydes 73 – 76

The racemic mixture of *trans*-hydroxyaldehydes **73**+**74** were separated by enzyme-mediated acylation with lipase AK (*Pseudomonas fluorescens*) in refluxing pentane in the presence of vinyl acetate within 48 h (Scheme 25). Similarly, the *cis*-hydroxyaldehydes **75+76** were separated under the same conditions.



Scheme 25. Separation of optically pure C_{15}-hydroxyaldehydes **73** – **76** by enzyme-mediated acylation.

While hydroxyaldehyde **74** was acylated to acetoxyaldehyde **117**, hydroxyaldehyde **73** remained unreacted. Due to the large difference in their solubility properties, **117** and **73** were readily separated by column chromatography and the latter was obtained in 94% *ee*. Acetoxyaldehyde **117** was nearly quantitatively hydrolyzed with KOH/MeOH at 0°C to hydroxyaldehyde **74** (93% *ee*). Similarly, *cis*-hydroxyaldehydes **75** and **76** were resolved by enzyme-mediated acylation with immobilized lipase AK (*Pseudomonas fluorescens*) in refluxing pentane in the presence of vinyl acetate in 50 h. Hydroxyaldehyde **75** underwent acylation to acetoxyaldehyde **118** while hydroxyaldehyde **76** remained unreacted (Scheme 25). Separation of **118** and **76** was readily accomplished by column chromatography. This

afforded (*3R,6S*)-3-hydroxy-α-ionylideneacetaldehyde (**76**) in 92% *ee*. Alkaline hydrolysis of **118** with KOH/MeOH at 0°C, provided (*3S,6R*)-3-hydroxy-α-ionylideneacetaldehyde (**75**) in 91% *ee*.

The course of these enzymatic acylation reactions were monitored by chiral HPLC (Eluent D, Appendix I) that allowed the separation of each enantiomeric pair (Figure 8). It should be pointed out that enantiomeric aldehydes **75** (HPLC peak 1) and **76** (HPLC peak 3) were well separated by chiral HPLC and this was also the case with aldehydes **73** (HPLC peak 2) and **74** (HPLC peak 4) (Figure 8). However, attempt to simultaneously separate these aldehydes resulted in partial separation between diastereomeric aldehydes.

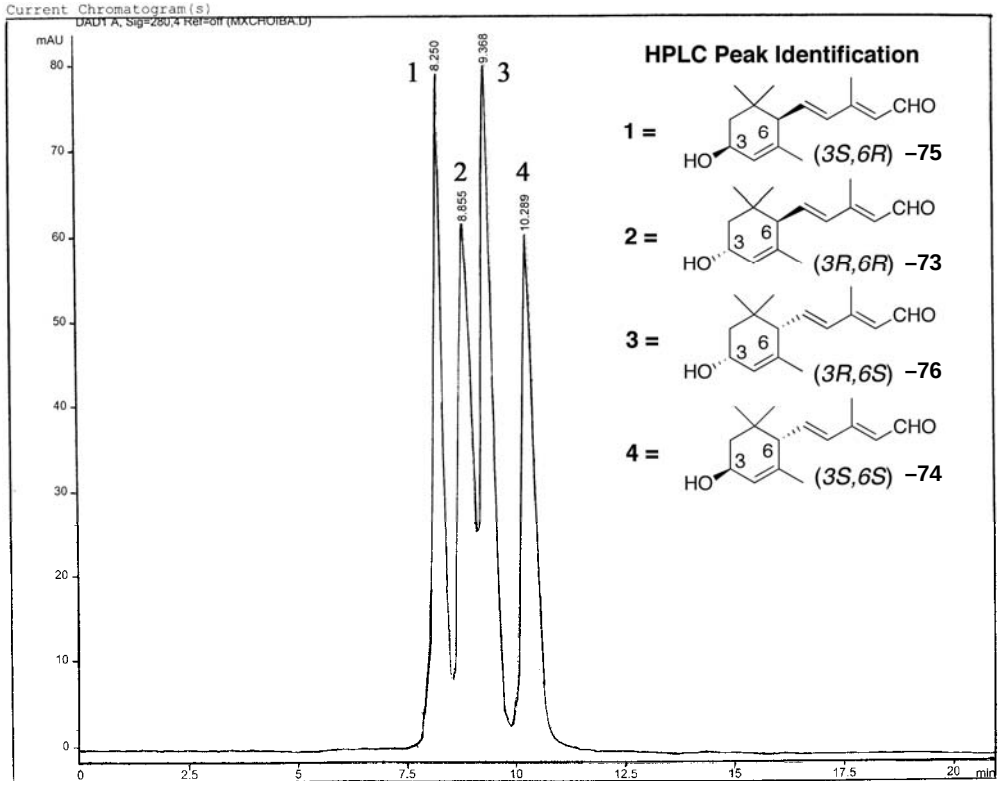


Figure 8. HPLC separation of hydroxyaldehydes **73** – **76** on a Chiralpak AD column (Eluent D, Appendix I).

Therefore, all four hydroxyaldehydes **73** – **76** became accessible in optical purities ranging from 91-94%. Before using these hydroxyaldehydes in the synthesis of the stereoisomeric luteins **1** – **4**, the absolute configuration of these aldehydes had to be established.

Determination of Absolute Configuration of Hydroxyaldehydes 73 – 76

Hydroxyldehydes **73** – **76** were fully characterized from their ^{1}H- and ^{13}C-NMR as well as MS and UV spectra. The relative stereochemistry of these aldehydes at C-3 and C-6 was established from comparison of the proton NMR chemical shifts of H-6 with published values for (*3R,3'R,6'R*)-lutein (**1**) and (*3R,3'S,6'R*)-lutein (**3**) (Figure 9).[77-80] It has been well documented that when H-6 and the hydroxyl group at C-3 are in a *cis*-geometry, the chemical shift of H-6 is shifted downfield by 0.25 ppm in comparison with the chemical shift of this proton when it is in a *trans*-geometry with OH at C-3.

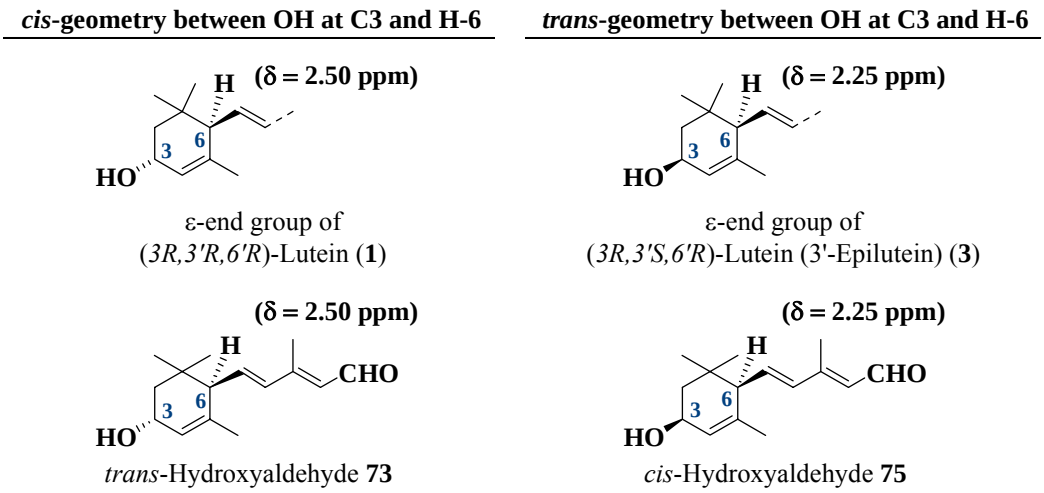
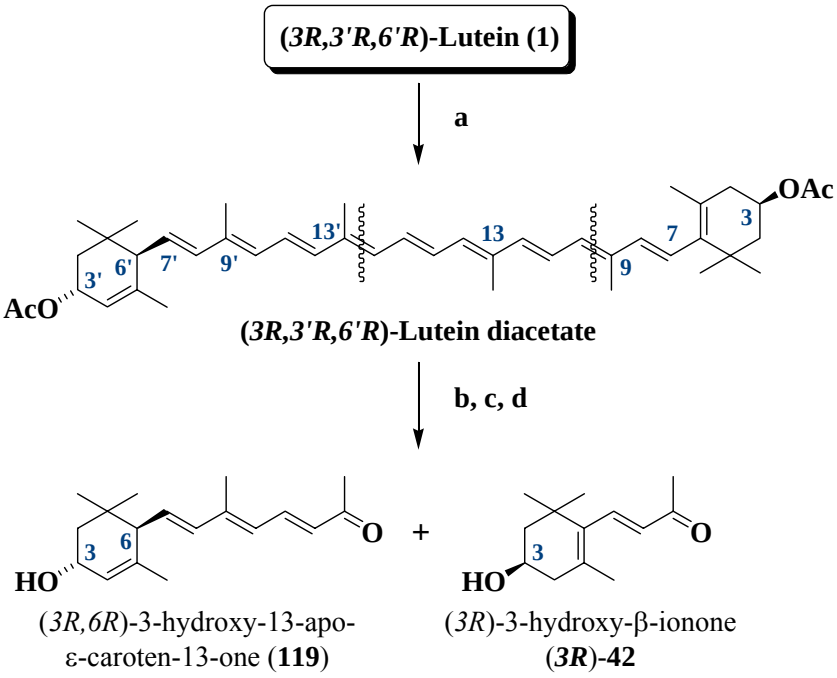


Figure 9. H-6' chemical shift in (*3R,3'R,6'R*)-lutein (**1**), (*3R,3'S, 6'R*)-lutein (**3**) and aldehydes **73** and **75**.

The chemical shift of H-6 proton in *trans*-aldehydes (**73** and **74**) in which this proton is in a *cis*-geometry with the hydroxyl group at C-3 appeared at δ = 2.50 ppm while this chemical shift in *cis*-aldehydes (**75** and **76**) (H-6 & OH in *trans*-geometry) moved upfield to δ = 2.25 ppm. Therefore, the relative stereochemistry of hydroxyldehydes **73** – **76** was assigned on this basis. However, the absolute configuration of these aldehydes could only be unequivocally determined with comparison of the circular dichroism (CD) data with a model compound in which the stereochemistry at C3 and C6 was known. We prepared this model compound by oxidative cleavage of the polyene chain of naturally occurring (*3R,3′R,6′R*)-lutein (**1**) in which the stereochemistry in the ε-end group of this carotenoid at C3' and C6' is known to be *R* (Scheme 26).[81-84]



Scheme 26. Oxidative degradation of (*3R,3′R,6′R*)-lutein (**1**). (a) Ac_2O, pyridine, THF, 50 °C, 98%; (b) TBHP, bleach (5.25% NaOCl), EtOAc, 0 °C to R.T., 3 h, 20%; (c) KOH:MeOH (10%, wt:v), CH_2Cl_2, R.T., 2 h, 98%; (d) column chromatography (hexane:acetone, from 98:2 to 95:5) followed by semipreparative normal phase HPLC (Eluent E, Appendix).

Prior to oxidative cleavage of **1**, the hydroxyl groups were protected by acylation with Ac_2O-pyridine and the resulting (*3R,3'R,6'R*)-lutein diacetate was then oxidatized with TBHP/bleach. After saponification and column chromatography, HPLC analysis of the crude product showed the presence of unreacted **1** (80%) and a number of oxidation products. Among these, (*3R,6R*)-3-hydroxy-13-apo-ε- caroten-13-one (**119**) with an ε-end group and (*3R*)-3-hydroxy-β-ionone (***3R***)-**42** with a β-end group were the only major stable products (**119**: (***3R***)-**42** = 3:1). These were separated by semipreparative normal phase HPLC and were fully characterized from their NMR, MS, UV-Vis, and CD spectra.

The CD spectra of *trans*-aldehydes (*3R,6R*)-**73** [281 nm (+18 mdeg), 242 nm (-1.3 mdeg)] and (*3S,6S*)-**74** [281 nm (-13 mdeg), 242 nm (+1.0 mdeg)] in hexane:ether:methanol (10:3:1) with strong opposite Cotton effects clearly indicated that these aldehydes were enantiomeric. The absolute configuration of **73** was assigned as (*3R,6R*) by comparison of its CD spectrum with that of hydroxyketone (*3R,6R*)-**119** [320 nm (+10.6 mdeg)] that similar to this aldehyde showed a positive Cotton effect (Figure 10).

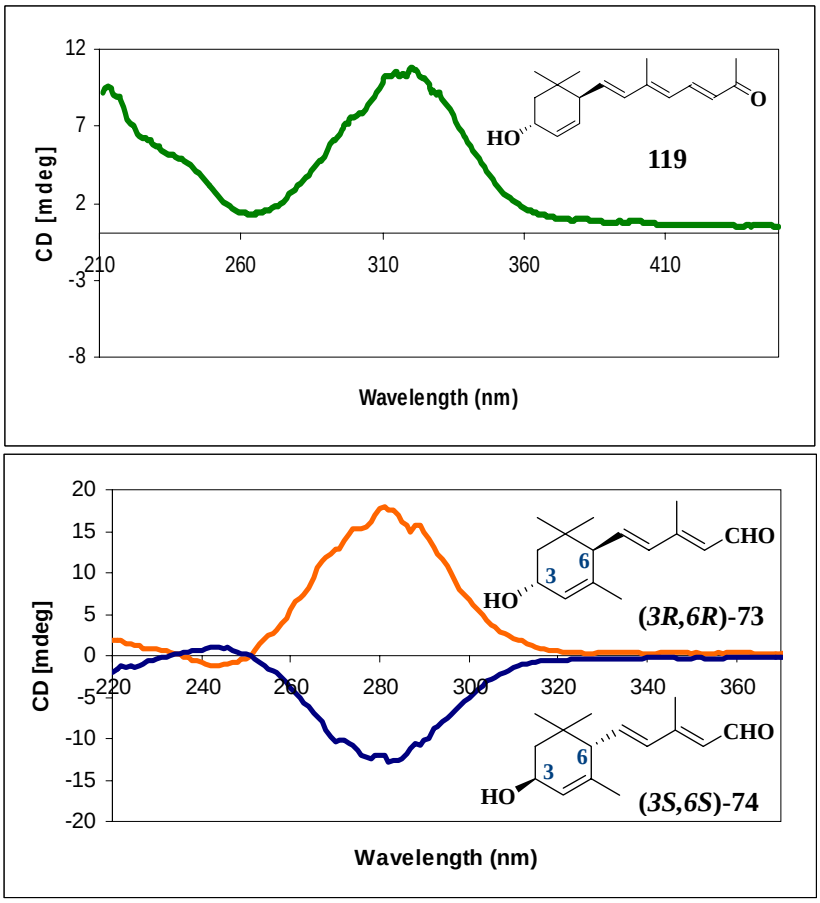
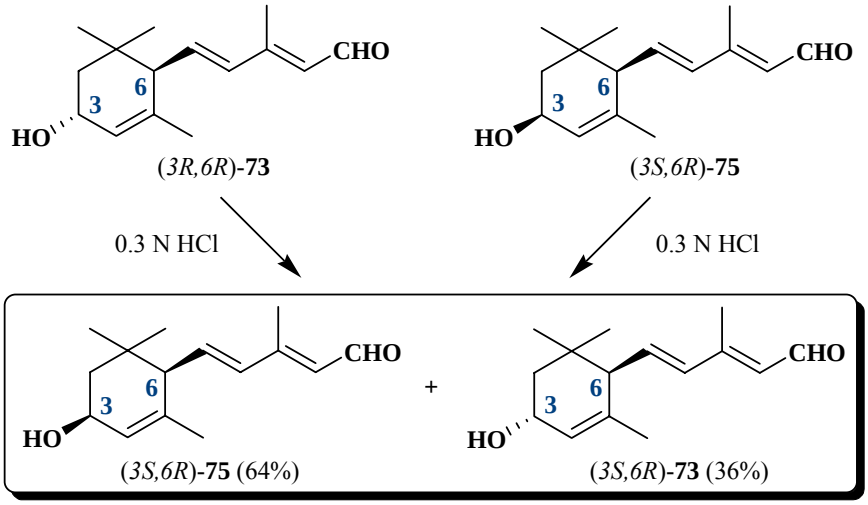


Figure 10. Circular dichroism (CD) spectra of C_{18}-ketone **119**, C_{15}-hydroxyaldehydes **73** and **74** in hexane:ether:MeOH = 10:3:1.

To establish the absolute configuration of **75** and **76**, aldehydes **73** and **75** were separately epimerized in dilute aqueous HCl similar to the previously reported epimerization of **1** to **3** (3'-epilutein).[40] Under identical reaction conditions, the HPLC analysis of the crude product revealed that hydroxyaldehydes **73** (36%) and **75** (64%) had reached an equilibrium that favored the *cis*-aldehyde **75** (Scheme 27).



Scheme 27. Epimerization reactions of hydroxyaldehydes **73** and **75** under acidic conditions.

The products of these reactions were then separated by semipreparative normal phase HPLC (Eluent A, Appendix I) and their absolute configurations were determined by NMR and CD. The H-6 chemical shift of the epimer of **73** appeared at δ = 2.25 ppm indicating a *trans*-geometry between H-6 and OH and on this basis, this epimer was identified as (*3S,6R*)-**75**. The CD spectrum of the epimer of **73** was also identical to the synthetic sample resolved by enzymatic acylation. In addition, the epimer of **75** was shown by NMR and CD to be identical to **73**. Because the CD spectra of (*3S,6R*)-**75** and **76** indicated that these aldehydes were enantiomeric, the absolute configuration of **76** was assigned as (*3R,6S*) (Figure 11).

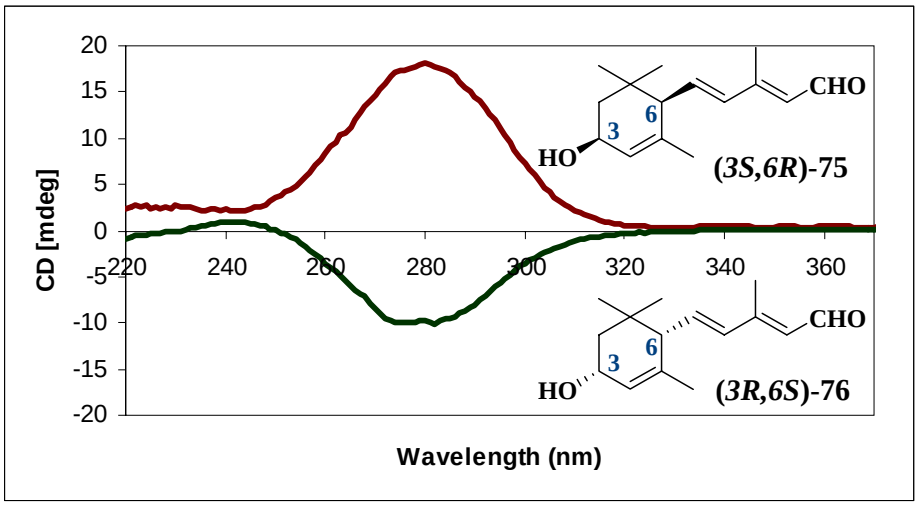
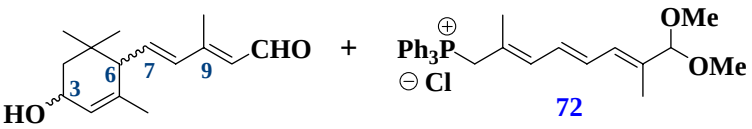


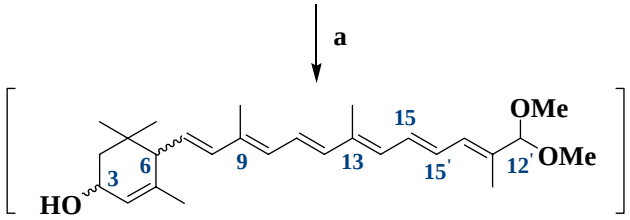
Figure 11. Circular dichroism (CD) spectra of C_{15}-hydroxyaldehydes **75** and **76** in hexane:ether:MeOH = 10:3:1.

Synthesis of Luteins 1 – 4 *via* C_{25}-Hydroxy-Apocarotenals 64 – 67

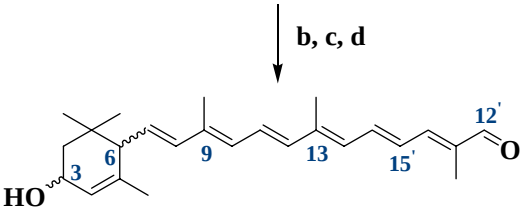
The C_{15}-hydroxyaldehydes **73** – **76** prepared in optical purities ranging from 91-94% were each first elongated to their corresponding protected C_{25}-aldehydes **68** – **71** by olefination with the protected Wittig salt **72** in the presence of NaOMe/MeOH at room temperature (Scheme 28).



73: (*3R,6R*), 94% *ee*; **74:** (*3S,6S*), 93% *ee*;
75: (*3S,6R*), 91% *ee*; **76:** (*3R,6S*), 92% *ee*



68: (*3R,6R)*, **69:** (*3S,6S*), **70:** (*3S,6R*), **71:** (*3R,6S*)



3-Hydroxy-12'-apo-ε-caroten-12'-al

64: (*3R,6R)*, 94% *ee*; **65:** (*3S,6S*), 93% *ee*;
66: (*3S,6R*), 91% *ee*; **67:** (*3R,6S*), 92% *ee*

Scheme 28. Synthesis of C_{25}-hydroxyaldehyde **64** – **67** from C_{15}-hydroxyaldehydes **73** – **76**. (a) NaOMe, MeOH, R.T., 4 h, 90% conversion by HPLC; (b) 0.3 N HCl, R.T., 1 h; (c) $Pd(OAc)_2$, EtOAc, reflux, 2h; (d) chromatography (hexane:EtOAc, 95:5 to 80:20), 53-85% isolated yield after chromatography.

After solvent evaporation and without isolation of the products, acetals **68** – **71** that were obtained as a mixture of *all-E* and 11*Z* were deprotected in dilute aqueous HCl (0.3 N) in acetone to give C_{25}-aldehydes **64** – **67** as a mixture of *all-E* and 11*Z*. Thus, prior to purification, the crude mixture of *all-E* and *11Z* isomers of C_{25}-aldehydes **64** – **67** were catalytically isomerized to their corresponding *all-E*-isomers in the presence of palladium (II) acetate in refluxing ethyl acetate within 2 h. In the following step, the individual aldehydes were purified by column chromatography to afford **64** – **67** in isolated yields ranging from 53-85%.

Under the conditions employed for the deprotection of acetals **68** – **71**, the hydroxyl group at C3 did not undergo epimerization under acidic condition and the optical purities of the resulting C_{25}-aldehydes **64** – **67** were not compromised. This was confirmed by chiral HPLC (Eluent D, Appendix I) of the individually synthesized C_{25}-aldehydes (Figure 12).

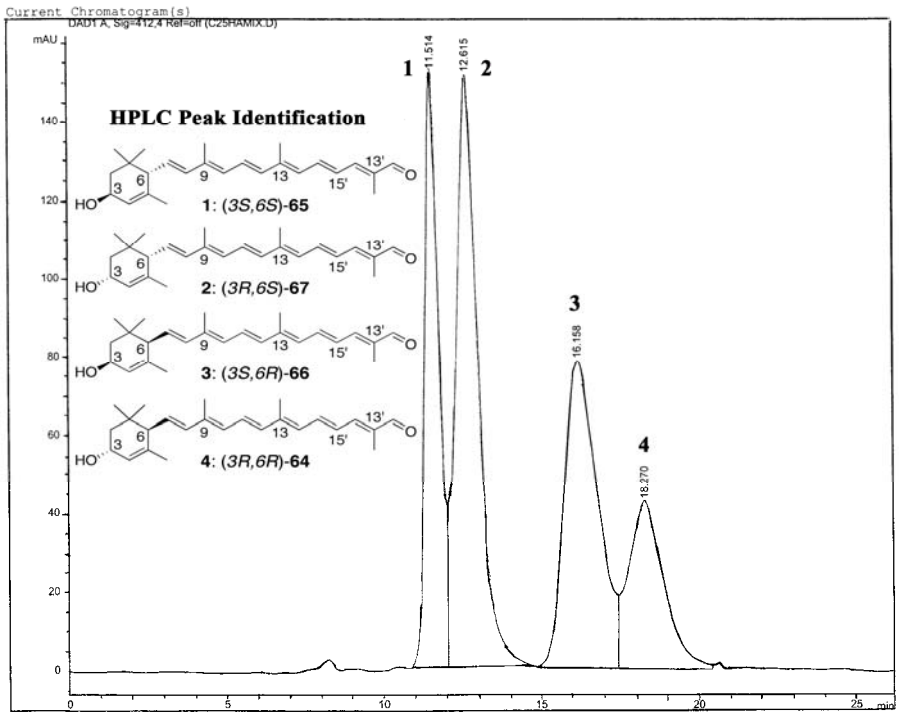


Figure 12. HPLC separation of 3-hydroxy-12'-apo-ε-caroten-12'-al (**64** – **67**) on a Chiralpak AD column (Eluent D, Appendix I).

It should be noted that enantiomeric C_{25}-aldehydes **65** (HPLC peak 1) and **64** (HPLC peak 4) were well separated by chiral HPLC and this was also the case with aldehydes **67** (HPLC peak 2) and **66** (HPLC peak 3) (Figure 13). However, attempt to simultaneously separate these aldehydes resulted in partial separation between diastereomeric aldehydes.

The CD spectra of **64** and **65** showed an opposite Cotton effect indicating that these aldehydes were enantiomeric. This was also the case with the CD spectra of **66** and **67** (Figure 18).

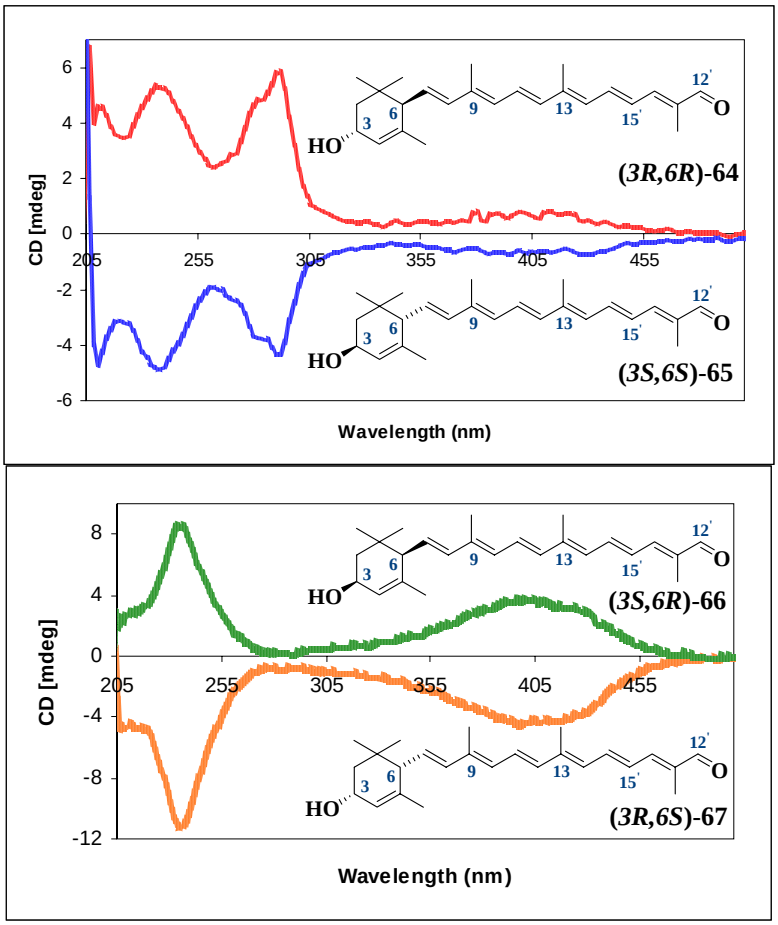
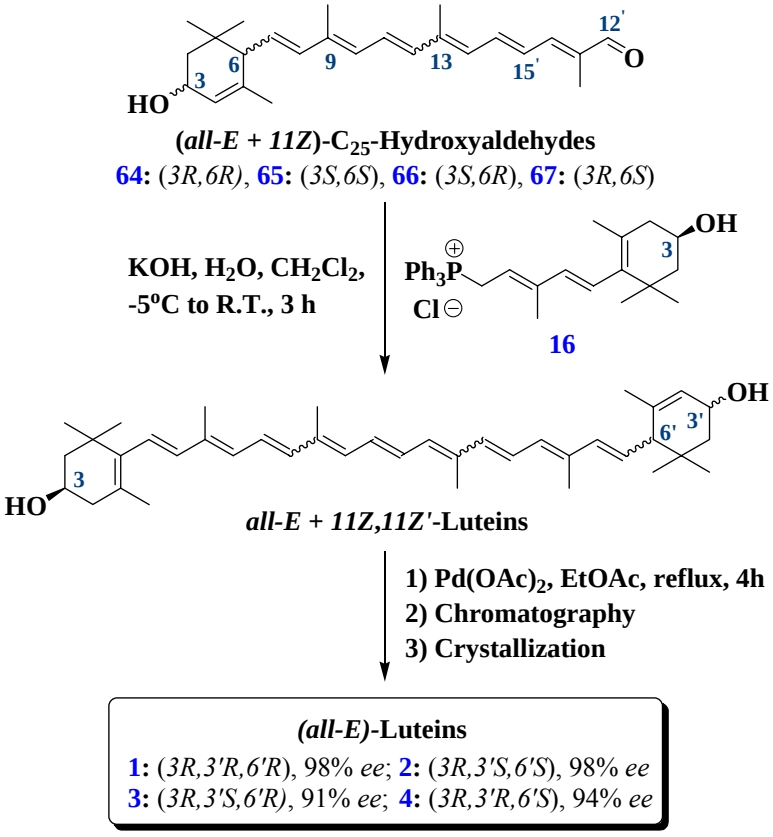


Figure 13. CD spectra of C_{25}-hydroxyaldehydes **64** – **67** in hexane:ether:MeOH = 10:3:1.

As described earlier, C_{25}-hydroxyaldehydes were initially obtained as a mixture of *all-E* and *11Z* isomers. Although the *11Z*-isomers were readily transformed into their corresponding *all-E*-isomers, this step was shown to be unnecessary and the

isomerization of the 11*Z* and 11'*Z*-bonds that are formed by Wittig coupling reactions could be postponed until after luteins **1** – **4** were prepared.

In the final step of the synthesis of luteins **1** – **4**, each of the C_{25}-hydroxyaldehydes **64** – **67** (*all-E* + 11*Z* or pure *all-E*) were allowed to react with the Wittig salt **16** [44-45] to yield their corresponding luteins **1** – **4** as a mixture of *all-E* and *Z*-isomers (Scheme 29).



Scheme 29. Final steps of the synthesis of luteins **1** – **4** *via* C_{25}-hydroxyaldehydes **64** – **67**.

The individually prepared *E*/*Z*-luteins **1** – **4** were then catalytically isomerized to their corresponding *all-E* isomers with $Pd(OAc)_2$ in a refluxing solution of ethyl acetate within 4 h. The isolated yields of *all-E*-luteins **1** – **4** after column chromatography and crystallization from hexane:acetone = 4:1 were in the range of

53-85% (91-98% *de*). It is interesting to note that the HPLC separation of diastereomeric (*3R,3'R,6'R*)-lutein (**1**) and (*3R,3'S,6'S*)-lutein (**2**) could not be achieved on a regular nitrile bonded column. This was presumably because of the remote position of the (*3R*)-hydroxyl group at C3 of these carotenoids that resulted in almost identical chromatographic properties for these luteins as though these were enantiomeric. This was also the case with diastereomeric (*3R,3'S,6'R*)-lutein (**3**) and (*3R,3'R,6'S*)-lutein (**4**). Therefore, luteins **1** – **4** had to be separated by chiral HPLC under two different sets of conditions to determine their diastereomeric excess. The HPLC separation of synthetic standards of luteins **1** and **2** is shown in Figure 14a (Eluent G) and that of standards of luteins **3** and **4** is shown in Figure 14b (Eluent H). The CD spectra of optically pure luteins **1** – **4** are shown in Figure 15.

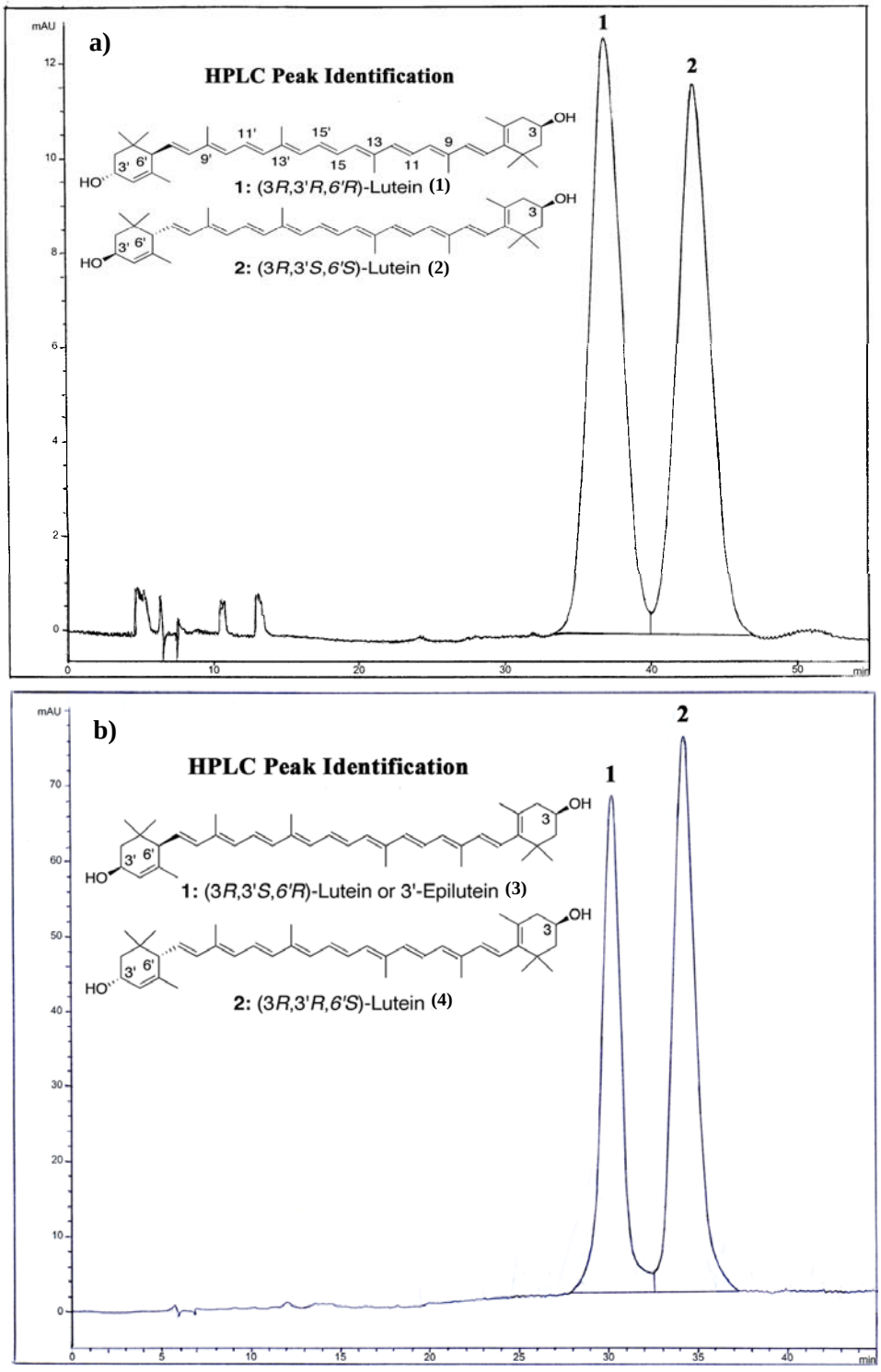


Figure 14. Chiral HPLC separation of luteins **1** – **4**. a) HPLC separation of standards of luteins **1** and **2** (Eluent G), b) HPLC separation of standards of luteins **3** and **4** (Eluent H); both separation were carried out on a Chiralpak AD column (Appendix I).

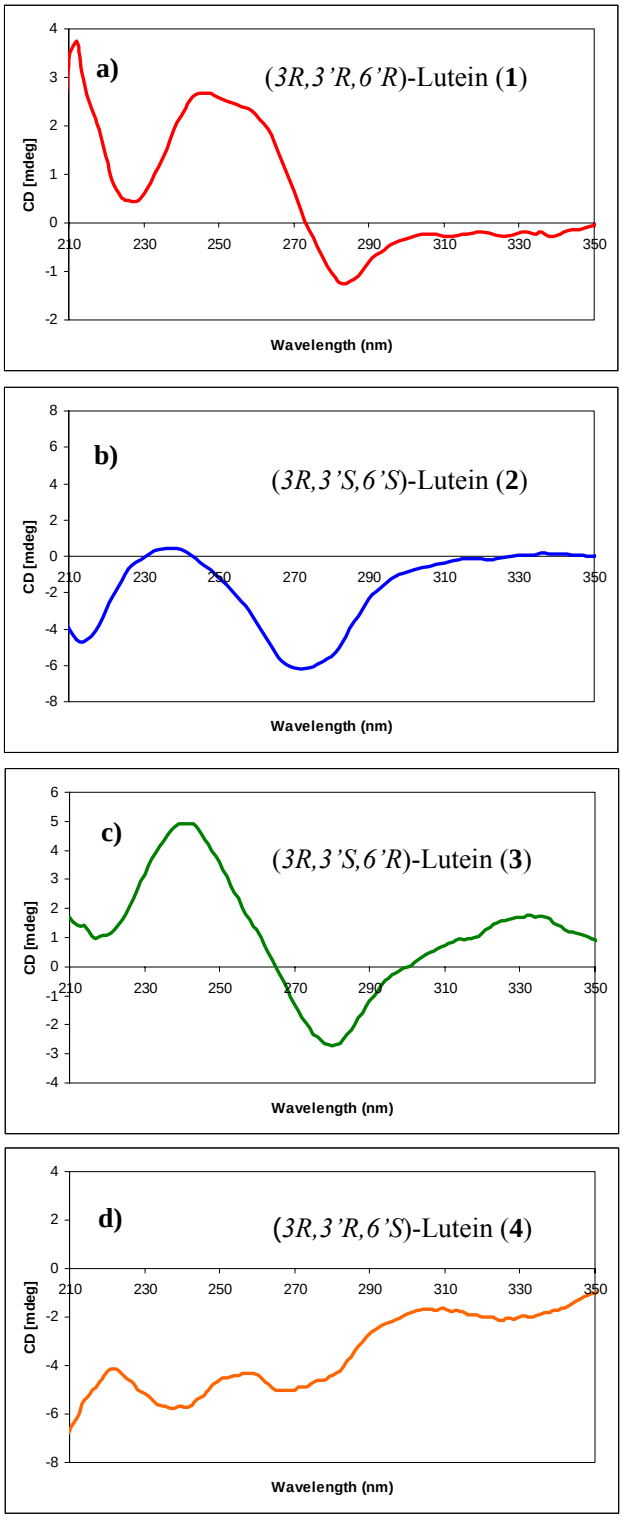


Figure 15. CD spectra (hexane:ether:MeOH = 10:3:1) of luteins **1** – **4**. a) (*3R,3'R,6'R*)-lutein (**1**); b) (*3R,3'S,6'S*)-lutein (**2**); c) (*3R,3'S,6'R*)-lutein (**3**) and d) (*3R,3'R,6'S*)-lutein (**4**).

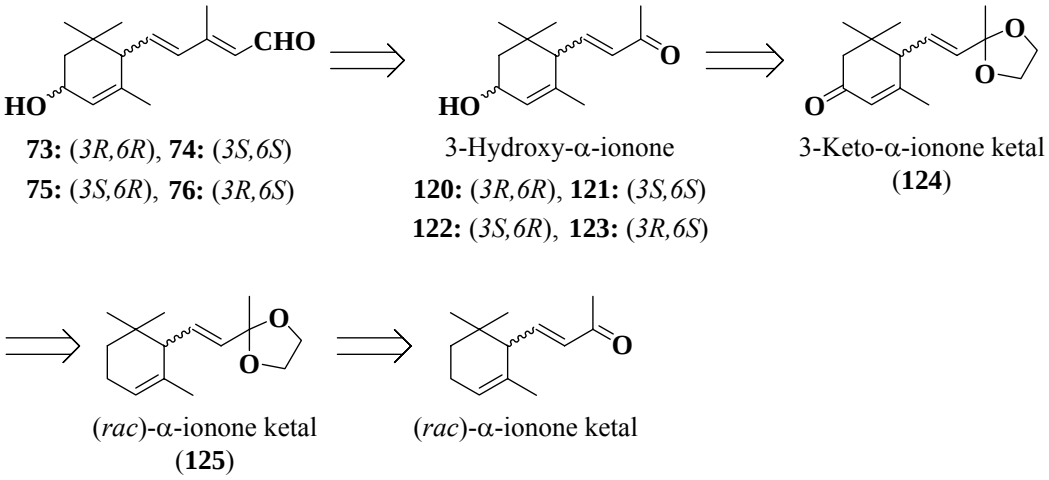
The overall yield of luteins **1** – **4** according to our synthetic strategy can be determined based on the stereochemistry of the targeted lutein. For example, if the synthesis of (*3R,3'R,6'R*)-lutein (**1**) and (*3R,3'S,6'S*)-lutein (**2**) is of particular interest, the one-pot reduction of ketonitrile **81** with potassium tri-*sec*-butylborohydride (K-Selectride®) followed by DIBAL-H would be the preferred route. This is because this reduction predominantly provides the *trans*-hydroxyaldehydes **73** and **74** that are precursors to the ε-end group of luteins **1** and **2**, respectively. Using this approach, the overall yields for luteins **1** and **2** from ketonitrile **81** were determined as follows: **81** → (**73** + **74**), 71% → **73** (30%) and **74** (31%) → **64** (26%) and **65** (28%) → **1** (18%) and **2** (20%). Because the isolated yield of ketonitrile **81** from (±)-α-ionone was 28% after crystallization, the overall yields of luteins **1** and **2** from (±)-α-ionone were 5% and 6%, respectively.

Similarly, if luteins **3** and **4** are the target carotenoids, the reduction of **81** with (*R*)-2-methyl-CBS-oxazaborolidine is preferred since this reagent provides the *cis*-hydroxyaldehydes **75** and **76** as the major products. According to this route, the overall yields of luteins **3** and **4** based on ketonitrile **81** were 17% and 19%, respectively. However, the calculated yields of **3** (5%) and **4** (6%) based on (±)-α-ionone were significantly lower.

While **81** served as the key starting material in our synthesis, the preparation and crystallization of this (*7E,9E*)-isomer from (±)-α-ionone in a low yield (28%) contributed to the low overall yield of luteins **1** – **4**.

Investigating an Alternative Route to C_{15}-Hydroxyaldehydes from (±)-α-Ionone

In the interest of atom economy, we decided to investigate an alternative apporoach to optically pure C_{15}-hydroxyaldehydes **73** – **76**. This involved synthesis of optically pure 3-hydroxy-α-ionones (**120** – **123**) prior to elongation to C_{15}-hydroxyaldehydes **73** – **76** according to the retrosynthetic sequence shown in Scheme 30.



73: (*3R,6R*), **74:** (*3S,6S*)
75: (*3S,6R*), **76:** (*3R,6S*)

3-Hydroxy-α-ionone
120: (*3R,6R*), **121:** (*3S,6S*)
122: (*3S,6R*), **123:** (*3R,6S*)

3-Keto-α-ionone ketal (**124**)

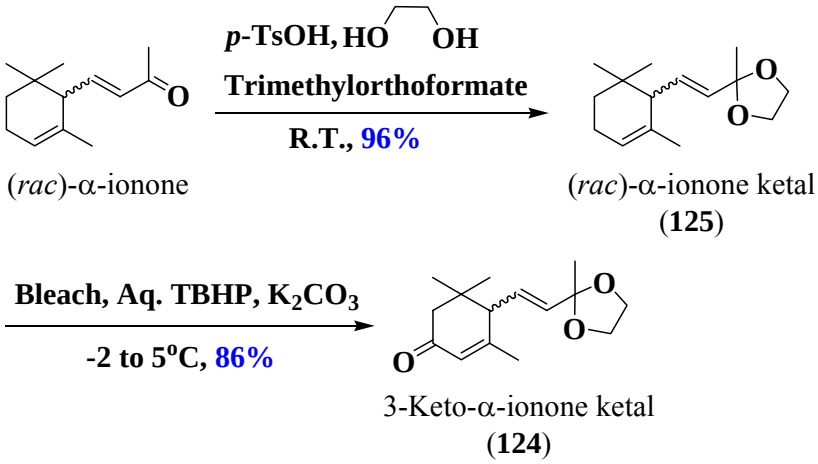
(*rac*)-α-ionone ketal (**125**)

(*rac*)-α-ionone ketal

Scheme 30. An alternative retrosynthetic route to C_{15}-hydroxyaldehydes **73** – **76**.

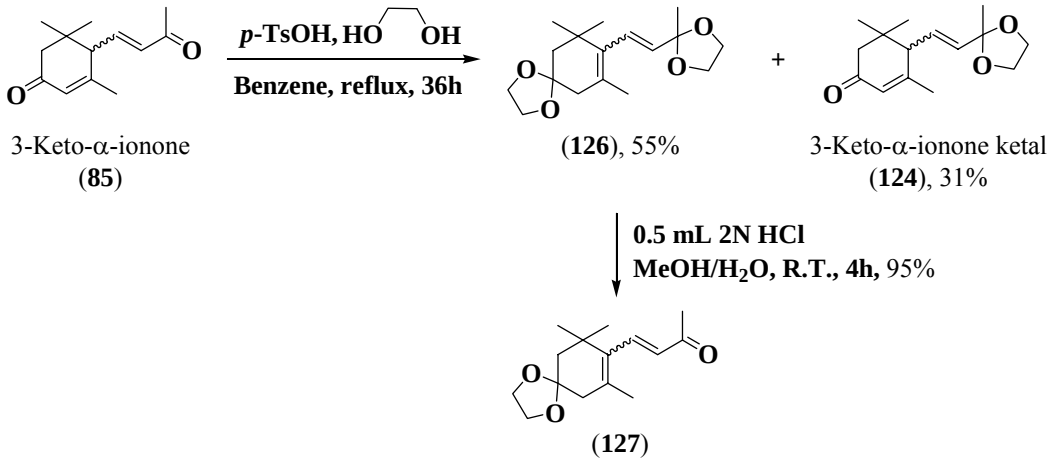
This idea was appealing because the stereochemistry of the precursor to **73** – **76** could be fixed at an earlier stage of the lutein synthesis. We rationalized that the optically pure 3-hydroxy-α-ionones (**120** – **123**) could be obtained by a similar strategy used previously for separating the optical isomers of C_{15}-ahydroxyaldehydes **73** – **76**. 3-Hydroxy-α-ionone (**124**) could be prepared in 4 convenient steps from the commercially available and inexpensive (±)-α-ionone *via* 3-keto-α-ionone ketal (**124**), and α-ionone ketal (**125**).

(±)-α-Ionone ketal (**125**) has been previously prepared from (±)-α-ionone by Pommer in 1958.[85] Following the Pommer's procedure, the carbonyl group of (±)-α-ionone was protected with ethylene glycol in the presence of trimethylorthoformate and catalytic amount of *p*-TsOH to afford (±)-α-ionone ketal (**125**) in nearly quantitative yield; this was used in the following step without purification (Scheme 31). Utilizing the bleach oxidation method described earlier (detailed mechanism is described in Scheme 20), 3-keto-α-ionone ketal (**124**) was obtained in 86% isolated yield after chromatography.



Scheme 31. Preparation of 3-keto-α-ionone ketal (**124**) from (±)-α-ionone.

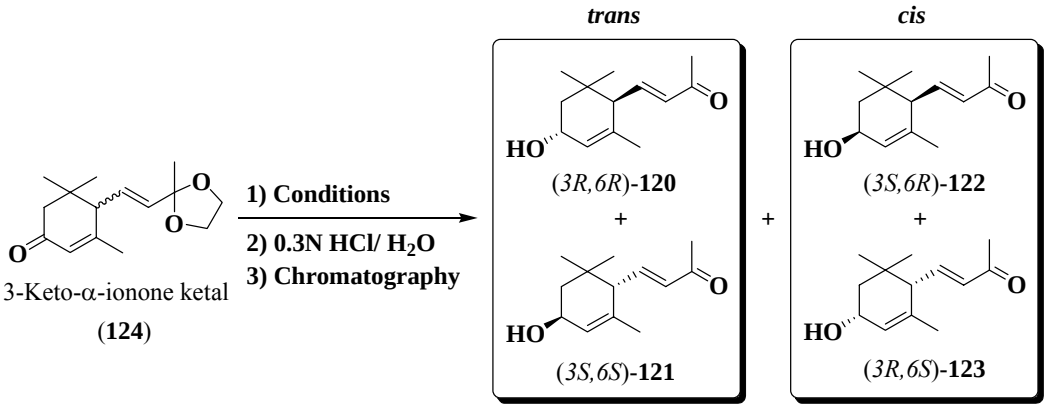
It should be pointed out that such a direct and efficient synthesis of keto ketal **124** has not been reported previously. However, this compound has been obtained as a side product in the synthesis of 3-(3,3-ethylenedioxy)-β-ionone (**127**) from 3-keto-α-ionone (**85**) (Scheme 32).[86]



Scheme 32. Preparation of ketoketal **127** from 3-keto-α-ionone (**85**) according to the method of Ellis et al.[86]

The reduction of 3-keto-α-ionone ketal (**124**) to 3-hydroxy-α-ionones (**120** – **123**) was carried out with a number of reducing agents to investigate the stereoselectivity of this reaction. These reduction reactions followed by deprotection gave two major products that were separated by column chromatography and fully characterized as (±)-*trans*-3-hydroxy-α-ionones (**120** and **121**) and (±)-*cis*-3-hydroxy-α-ionones (**122** and **123**). The results of these reactions are summarized in Table 4. The *trans* and *cis* isomers refer to the stereochemical relationship between the OH at C-3 and the enone side chain at C-6. Unfortunately, no significant stereoselectivity with various reagents could be achieved in these reduction reactions.

Table 4. Reduction of 3-keto-α-ionone ketal (**124**) to 3-hydroxy-α-ionone (**120** – **123**).



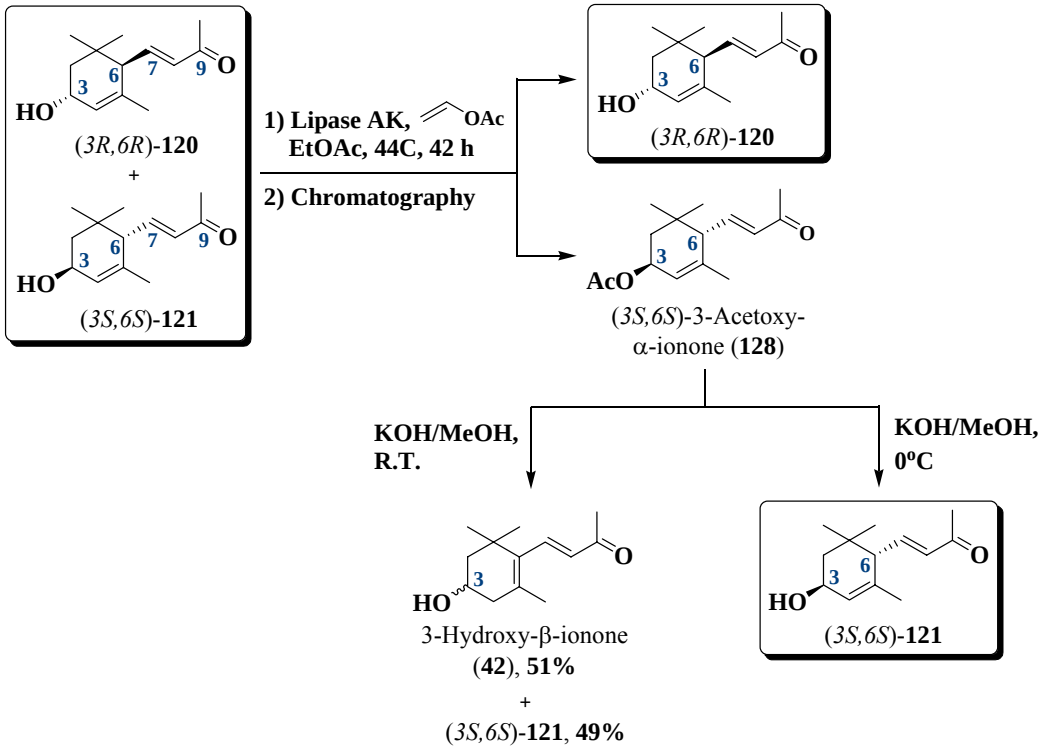
Entry	Conditions	(**120**+**121**):(**122+123**) (*trans:cis*)[a,b]	Conversion (%)
1	$NaBH_4$, EtOH	1.0 : 1.0	58
2	$NaBH_4$/*dl*-Tartaric acid (3/1), EtOH	1.0 : 1.0	77
3	$NaBH_4$-$CeCl_3$	1.0 : 1.9	40
4	9-Borabicyclo[3.3.1]nonane (9-BBN), THF	1.0 : 1.4	20
5	Red-Al™ = $NaAlH_2(OCH_2CH_2OMe)_2$, TBME	1.0 : 1.0	57
6	DIBAL-H, CH_2Cl_2	1.0 : 1.9	90
7	N-Selectride™ = $NaB[CHMeCH_2CH_3]_3H$, TBME	1.0 : 1.4	80
8	K-Selectride™ = $KB[CHMeCH_2CH_3]_3H$, TBME	1.2 : 1.0	85

[a] Indicates the stereochemical relationship between the hydroxyl group at C3 and the enone side chain at C6. [b]Ratios were determined by HPLC (Elent A, Appendix I)

Enzyme-Mediated Separation of (±)-3-Hydroxy-α-Ionone (120 – 123)

Initially we examined lipase AK since we had successfully employed this enzyme in separation of enantiomeric aldehydes **73** and **74** as well as **75** and **76**. In the following step, the purified racemic mixture of *trans*-hydroxy-α-ionones **120**+**121**

was subjected to enzyme-mediated acylation using vinyl acetate as an acyl donor. The course of enzymatic acylation of ionones **122**+**123** was followed by chiral HPLC (Eluent I, Appendix I). After 42h, the acylation became sluggish and HPLC analysis revealed that the unesterified ionones were present in a ratio of 10/1. The major unesterified product was tentatively identified as (*3R,6R*)-**120** and the structure of the acylated ionone was assigned as (*3S,6S*)-acetoxyionone **128** (Scheme 33). This assignment was based on our previous experience with the enzymatic separation of (*3R,6R*)-**73** and (*3S,6S*)-**74** that only resulted in acylation of the *3S* hydroxyl group.



Scheme 33. Enzyme-mediated acylation of racemic mixture of *trans*-hydroxyionones **120** and **121**.

Acetoxyionone **128** was readily separated from hydroxyionones **120** by column chromatography. Ionone **120** was shown by chiral HPLC to have an *ee* of 82%. Upon soaponification of 3-acetoxy-α-ionone (**128**) at room temperature, in addition to ionone **121** (49%), another product was formed which was separated by semipreparative HPLC (Eluent E, Appendix I) and fully characterized as 3-hydroxy-β-ionone (51%, **42**). This finding was interesting to us since **42** is an important precursor to Wittig salt **16** and has been employed in the synthesis of zeaxanthin.[42,43]

To avoid the formation of 3-hydroxy-β-ionone (**42**), the soaponification of 3-acetoxy-α-ionone (**128**) was carried out at 0°C to afford 3-hydroxy-α-ionone (**121**) quantitatively. Chiral HPLC revealed an *ee* of 28% for this ionone (Eluent I, Appendix I). These results indicated that lipase AK exhibits poor enantioselectivity towards ionones **120** and **121**. Therefore, we examined other commercially available enzymes such as lipase PS, lipase A, and lipase AY in an attempt to resolve (*3R,6R*)-**120** and (*3S,6S*)-**121**; the results of these enzymatic acylation are compared with lipase AK in Table 5.

Table 5. Enzyme-mediated acylation of a racemic mixture of *trans*-hydroxyionones **120** and **121**.

.

Entry	Conditions	**120** (% ee)	**121** (% ee)
1	Lipase AK, Vinyl acetate, EtOAc, 44°C, 42 h	82	28
2	Lipase PS (immobilized), Vinyl acetate, EtOAc, 50°C, 24 h	86	86
3	Lipase A (immobilized), Vinyl acetate, EtOAc, R.T to 50°C, 68 h	No Reaction	
4	Lipase AY (immobilized), Vinyl acetate, EtOAc, R.T to 50°C, 68 h	No Reaction	

The reaction with lipase PS was extremely slow and only 5% of **121** underwent acylation after 24 hours and addition of more enzyme did not alter the course of this reaction. To increase the rate of this enzymatic acylation, the separation of **120** and **121** was examined with immobilized lipase PS. With this enzyme, (*3R,6R*)-**120** (86% *ee*) and (*3S,6S*)-**121** (86% *ee*) were separated in moderate *ee*. The acylation with immobilized lipase A and lipase AY did not give any acylated product even after 68 hours.

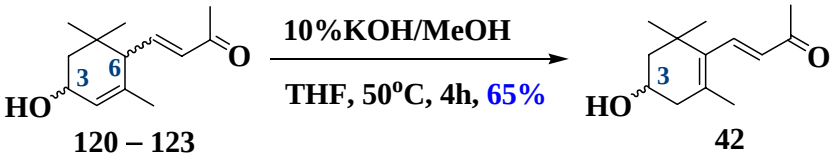
We then turned our attention to the enzyme-mediated separation of *cis*-hydroxyionones **122** and **123**. Unfortunately, we could not observe any acylation of these ionones with lipase AK, PS, A and AY under various experimental conditions. Because of theses disappointing results, the enzymatic separation of hydroxyionones was abandoned.

Despite the fact that the separation of these ionones by enzymatic acylation were unsuccessful, they lead us to a very intriguing observation that *trans*-acetoxyionone **128** can be isomerized to 3-hydroxy-β-ionone (**42**) under mild basic conditions. While the *trans*-hydroxyionones **120** and **121** underwent partial double bond isomerization during soaponification, it was unclear to us whether the *cis*-hydroxyionones **122** and **123** could do the same.

In such a case, all four stereoisomers of 3-hydroxy-α-ionones (**120** – **123**) could be transformed into a racemic mixture of 3-hydroxy-β-ionones with only one stereogenic center. Although we were unable to separate racemic mixture of 3-hydroxy-α-ionone by enzyme-mediated acylation, we rationalized that this approach

could be more successful with the separation of racemic mixture of 3-hydroxy-β-ionone.

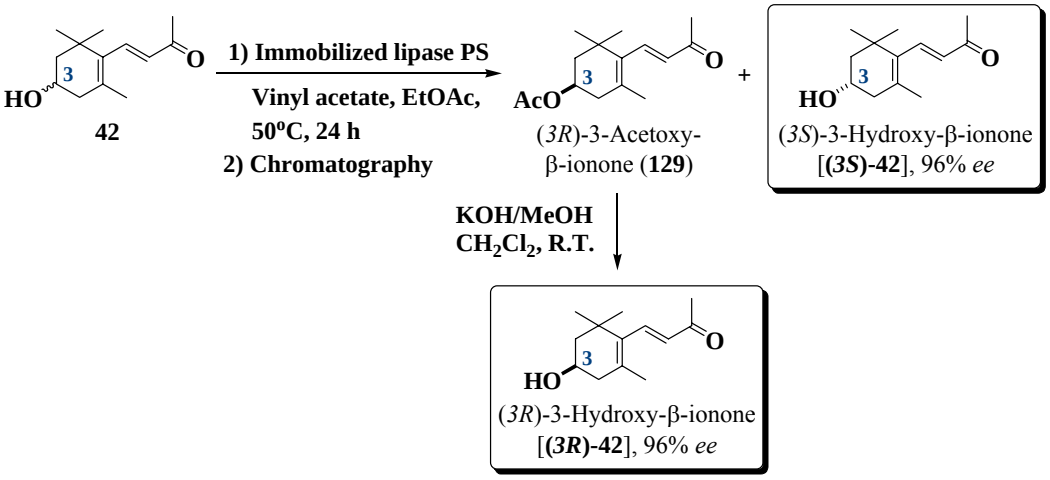
Therefore, the base-catalyzed double bond isomerization of 3-hydroxy-α-ionones (**120** – **123**) was further explored at elevated temperature to force the double bond isomerization of 3-hydroxy-α-ionones (**120** – **123**) to completion. After tuning the condition of this reaction, hydroxy-α-ionones **120** – **123** were converted to (±)-3-hydroxy-β-ionone (**42**) with KOH/MeOH (10%, wt/v) at 50 ^{o}C after 1 hour in THF. After purification by column chromatography, **42** was obtained in 65% isolated yield (Scheme 34).



Scheme 34. Double bond isomerization of 3-hydroxy-α-ionones **120** – **123** to (±)-3-hydroxy-β-ionone (**42**).

Enzyme-Mediated Acylation of (±)-3-Hydroxy-β-Ionone.

The racemic mixture of 3-hydroxy-β-ionone was resolved by enzyme-mediated acylation with immobilized lipase PS (*pseudomonas cepacia*) in EtOAc in the presence of vinyl acetate within 20 h at R.T (Scehem 35).



Scheme 35. Enzymatic acylation of (±)-3-Hydroxy-β-ionone (**42**).

While (*3R*)-3-hydroxy-β-ionone **(*3R*)-42** was acylated to (*3R*)-3-acetoxy-β-ionone (**129**), (*3S*)-3-hydroxy-β-ionone **(*3S*)-42** remained unreacted. Due to the large difference in their solubility properties, **129** and **(*3S*)-42** were readily separated by column chromatography. Acetoxyionone **129** was nearly quantitatively soaponified to hydroxyionone **(*3R*)-42** with KOH/MeOH at room temperature. According to chiral HPLC, (*3R*)-3-hydroxy-β-ionone [**(*3R*)-42**] and its (*3S*)-isomer [**(*3S*)-42**] were each obtained in 96% enantiomeric excess (*ee*). This was confirmed from the chiral HPLC profiles (Eluent J, Appendix I) of the individually separated enantiomers (Figure 16).

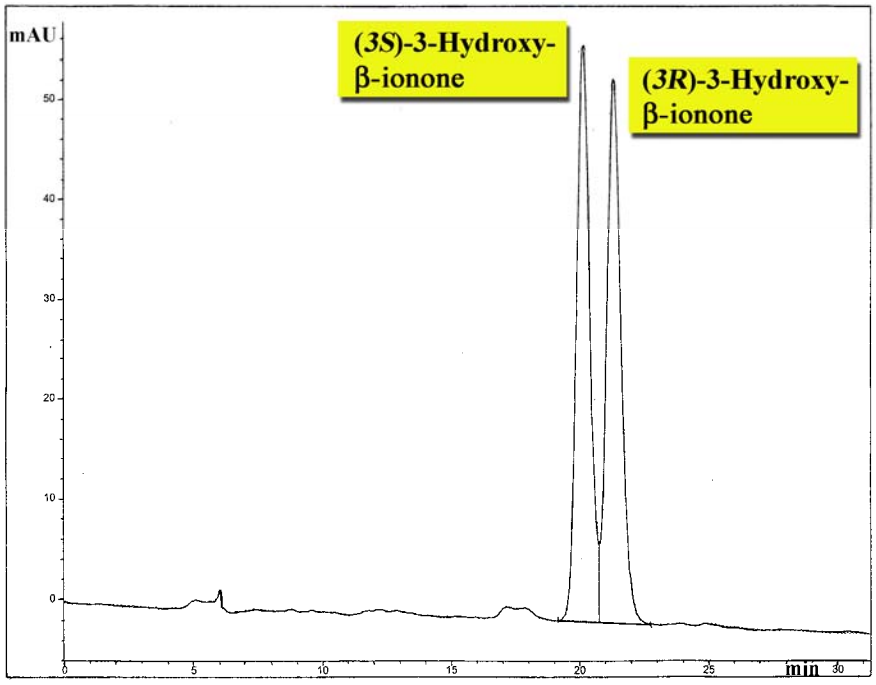


Figure 16. Chiral HPLC separation (Eluent J, Appedix I) of a racemic mixture of (*3R*)-3-hydroxy-β-ionone [**(*3R*)-42**] and (*3S*)-3-hydroxy-β-ionone [**(*3S*)-42**].

Similar results were also obtained by employing immobilized lipase AK (*pseudomonas fluorescens*). The absolute configuration of hydroxyionones **(*3R*)-42** and **(*3S*)-42** was determined by comparison of their CD spectra (Figure 17b) with a standard sample of (*3R*)-3-hydroxy-β-ionone (Figure 17a) as described earlier. This **(*3R*)-42** was obtained from oxidative cleavage of naturally occurring (*3R,3'R,6'R*)-lutein (**1**) (Scheme 26). CD spectra of **(*3R*)-42** and **(*3S*)-42** showed opposite Cotton effect and clearly indicated that these ionones were enantiomeric.

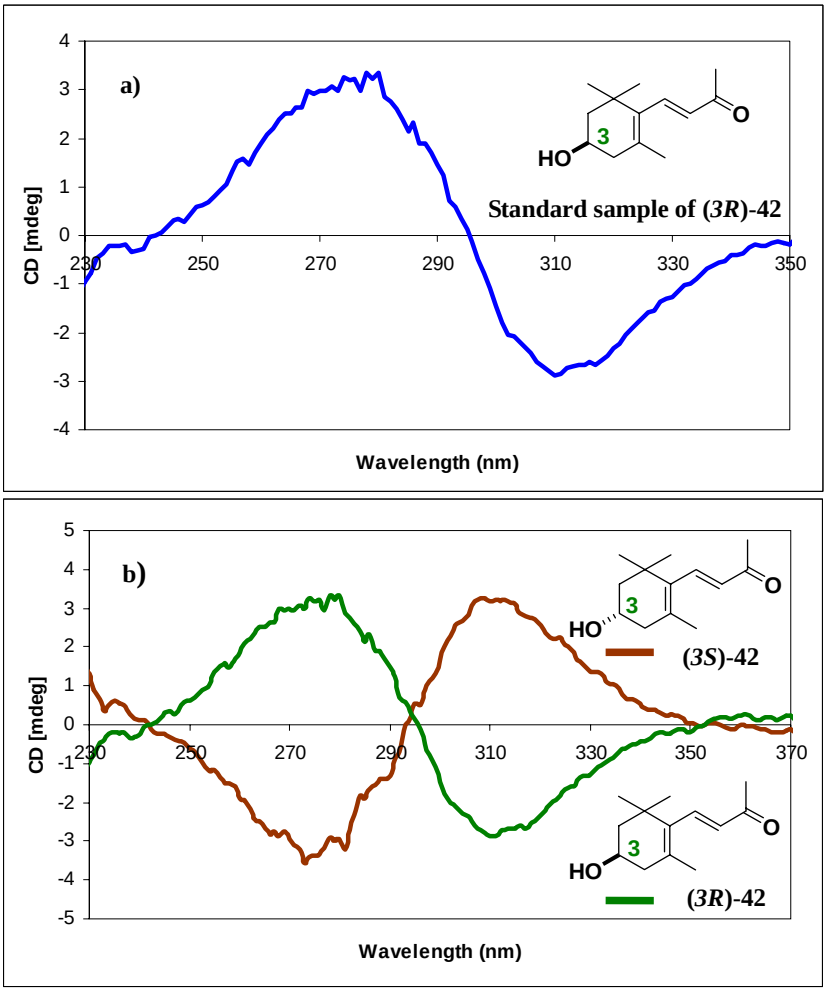
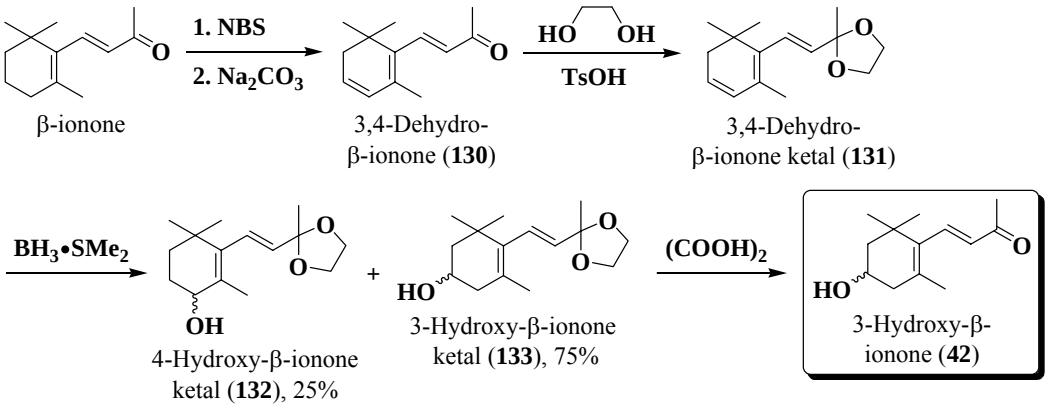


Figure 17. CD spectra (hexane:ether:methanol = 10:3:1) of 3-hydroxy-β-ionone, a) (*3R*)-3-hydroxy-β-ionone [**(*3R*)-42**] from oxidative degradation of (*3R,3'R,6'R*)-lutein (**1**) and b) (*3R*)-3-hydroxy-β-ionone [**(*3R*)-42**] and its (*3S*)-isomer [**(*3S*)-42**] separated by enzymatic acylation.

As described earlier in the introduction section (page 17-19), the synthesis of 3-hydroxy-β-ionone (**42**) has been reported as a racemic mixture by Loeber et al.[42] and as single enantiomers [**(*3R*)-42** and **(*3S*)-42**] by Rüttimann and Mayer[43]. Both of these investigators used two completely different approaches than the one we described

above. In 1992, Broom et al. also reported on the synthesis of 3-hydroxy-β-ionone from β-ionone according to the reaction pathways shown in Scheme 36.[87]

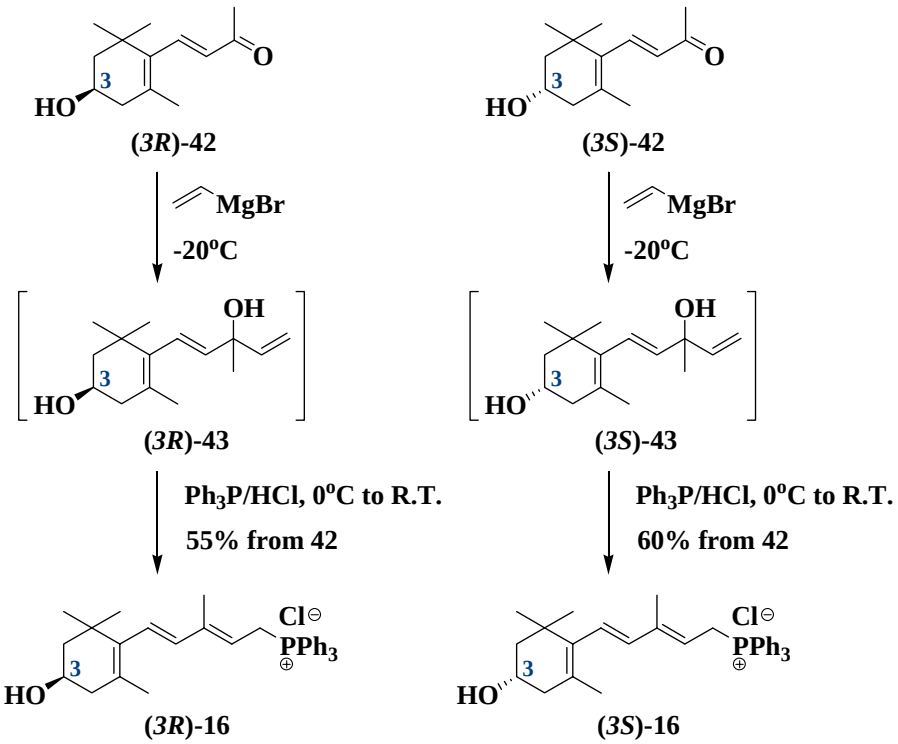


Scheme 36. Synthesis of 3-hydroxy-β-ionone according to the method of Broom et al.[87]

The key starting material for this synthesis was 3,4-dehydro-β-ionone (**130**) that was prepared from β-ionone according to a published procedure.[88] The ketone **130** was then protected as a 1,3-dioxolane to yield 3,4-dehydro-β-ionone ketal (**131**) that was hydroborated with borane-dimethyl sulfide to afford (±)-3-hydroxy-β-ionone ketal (**132**, 75%) and (±)-4-hydroxy-β-ionone ketal (**133**, 25%). Ketal **133** was isolatd from the mixture in 32% yield and was subsequently deprotected with oxalic acid to afford **42**. However, the authors were unable to resolve the racemic mixture of ionone **42**.

Transformation of (*3R*)-3-Hydroxy-β-Ionone [(*3R*)-42] and its *3S*-Isomer [(*3S*)-42] to (*3R,3'R*)-Zeaxanthin (5) and (*3S,3'S*)-Zeaxanthin (6)

We used the reported procedure by Rüttimann and Mayer to separately transform (*3R*)-3-hydroxy-β-ionone [**(*3R*)-42**] and its *3S*-isomer [**(*3S*)-42**] to the Wittig salts 3-hydroxy-(β-ionylideneethyl)triphenylphosphonium chlorides **(*3R*)-16** and **(*3S*)-16** *via* vinyl-α-ionols **(*3R*)-43** and **(*3S*)-43**, respectively. (Scheme 37).[43]



Scheme 37. Transformation of (*3R*)-3-hydroxy-β-ionone [**(*3R*)-42**] and its *3S*-isomer [**(*3S*)-42**] to Wittig salts **(*3R*)-16** and **(*3S*)-16** according to the method of Mayer and Rüttimann.[43]

The crude ionols **(*3R*)-43** and **(*3S*)-43** were directly converted to Wittig salts **(*3R*)-16** and **(*3S*)-16** without purification. After work-up, the crude Wittig salts **(*3R*)-16** was crystallized from 1,2-dichloroethane and ethyl acetate at -20°C to afford **(*3R*)-**

16 in 60% yield as a grayish powder. Similarly, **(*3S*)-16** was crystallized using the same solvent system to afford this Wittig salt in 55% yield. The absolute configuration of the Wittig salts **(*3R*)-16** and **(*3S*)-16** was confirmed by comparison of their CD spectra with a standard sample of Wittig salt **(*3R*)-16** obtained from DSM Nutritional Products (Basel, Switzerland) (Figure 18).

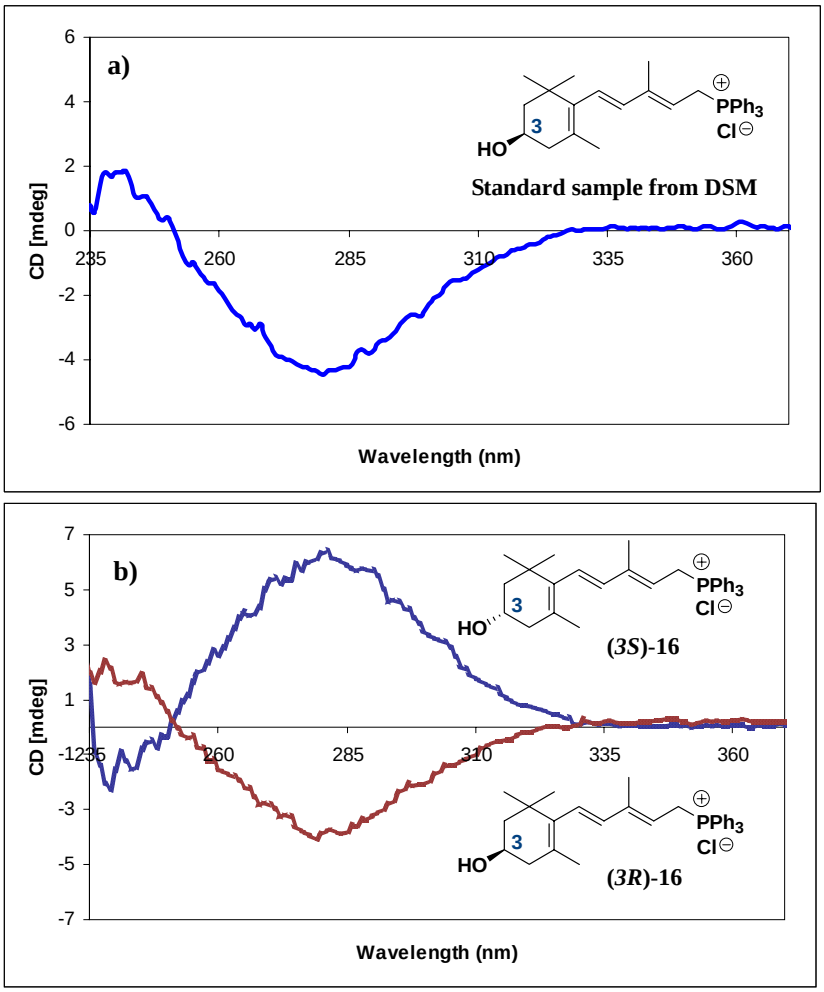
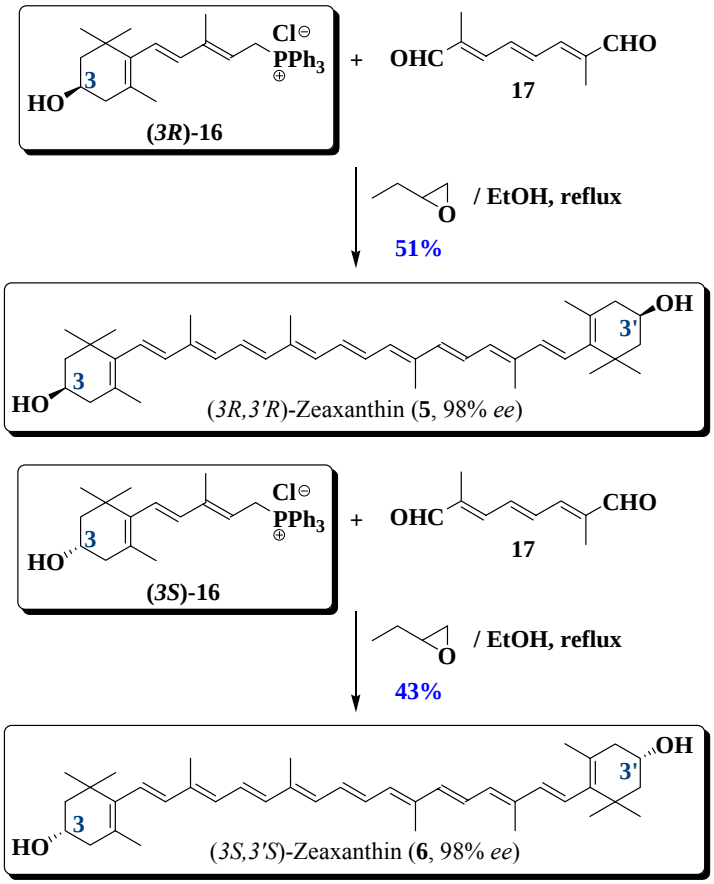


Figure 18. CD spectra (hexane:ether:methanol = 10:3:1) of Wittig salt **16**, a) a standard sample of (*3R*)-3-hydroxy-(β-ionylideneethyl)triphenylphosphonium chloride obtained from DSM Nutritional Products (Basel, Switzerland) and (b) individually prepared Wittig salts **(*3R*)-16** and **(*3S*)-16**.

The final step of the synthesis of (*3R,3′R*)-zeaxanthin (**5**) and (*3S,3′S*)-zeaxanthin (**6**) was accomplished according to the method of Widmer et al.[44] These were each prepared in 98% *ee* by double Wittig condensation of C_{10}-dialdehyde **17** with Wittig salts **(*3R*)-16** and **(*3S*)-16** in 51% and 43% isolated yields after column chromatography and low temperature crystallization, respectively (Scheme 38). 1,2-Epoxybutane in these reactions served as acid scavenger. The ^{1}H and ^{13}C NMR chemical shifts of (*3R,3′R*)-zeaxanthin (**5**) and (*3S,3′S*)-zeaxanthin (**6**) were in agreement with the published data for these carotenoids.[88]



Scheme 38. Synthesis of (*3R,3′R*)- zeaxanthin (**5**) and (*3S,3′S*)-zeaxanthin (**6**) from Wittig salts **(*3R*)-16** and **(*3S*)-16**, respectively.

These reactions were carried out on several hundred milligrams scale and our yields were significantly lower than the reported 90% yield by Widmer et al. who performed this coupling reaction on kilogram scale. This may be due to the fact that Widmer et al. used only 300 mL of EtOH per 1 Kg of the Wittig salt to prevent *E*/*Z* isomerization of *all-E*-zeaxanthin. When we carried out this reaction on a small scale, we had to use relatively large volume of solvent to be able to control the temperature. Consequently, at reflux temperature of EtOH, *E/Z* isomerization could have been responsible for our lower yield since *Z*-carotenoids do not crystallize well in comparison with their *all-E* counterparts.

The optical purity of **5** and **6** was determined by chiral HPLC (Eluent L, Appendix I) that not only allowed the separation of these optical isomers but it also separated the optically inactive (*3R,3'S;meso*)-zeaxanthin (**7**) (Figure 19). As shown in the CD spectra of (*3R,3'R*)-zeaxanthin (**5**) and (*3S,3'S*)-zeaxanthin (**6**) in Figure 20, these enantiomeric carotenoids exhibited opposite Cotton effect. The absolute configuration of (*3R,3'R*)-zeaxanthin was assigned by comparison of its CD spectrum with a standard sample of this carotenoid donated by the DSM Nutritional Products (Basel, Switzerland).

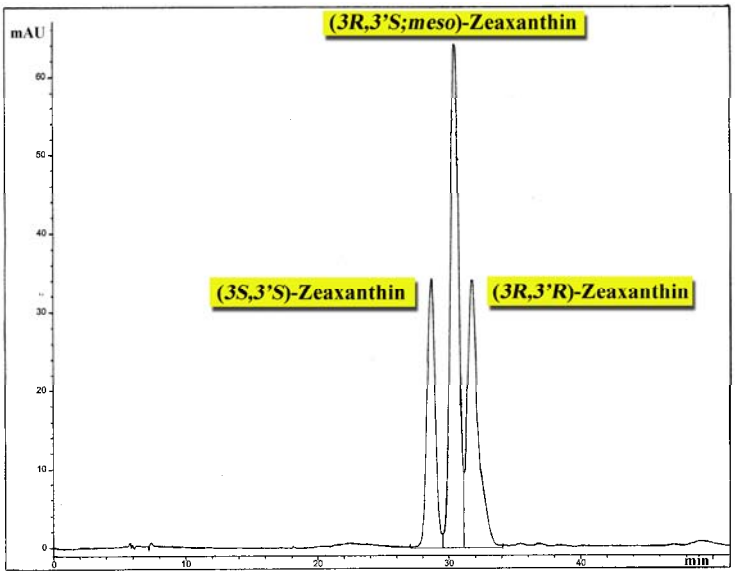


Figure 19. Chiral HPLC (Eluent L, Appendix I) separation of a mixture of (*3R,3'R*)-zeaxanthin (**5**), (*3S,3'S*)-zeaxanthin (**6**), and (*3R,3'S;meso*)-zeaxanthin (**7**).

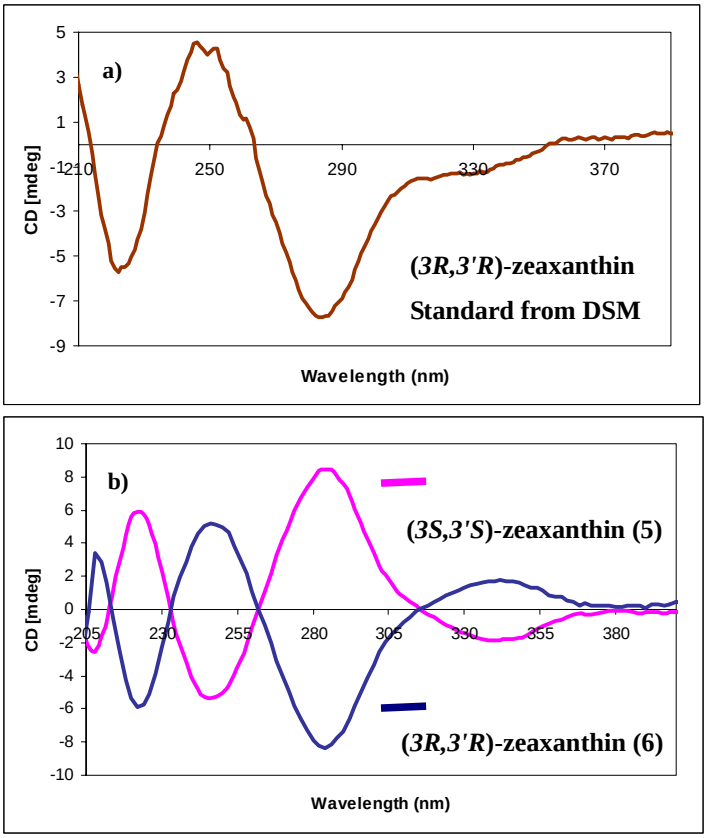


Figure 20. CD spectra (hexane:ether:methanol = 10:3:1) of zeaxanthins, a) a standard sample of (*3R,3'R*)-zeaxanthin (**5**) from DSM Nutritional Products (Basel, Switzerland) and b) individually prepared (*3R,3'R*)-zeaxanthin (**5**) and (*3S,3'S*)-zeaxanthin (**6**).

The overall yields for zeaxanthins **5** and **6** from (±)-α-ionone were determined as follows: (±)-α-ionone → **125** (96%) → **124** (83%) → **120** – **123** (74%) → [**(*3R*)-42** + **(*3S*)-42**] (48%) → **(*3R*)-42** (22%) and **(*3S*)-42** (21%) → **(*3R*)-16** (12%) and **(*3S*)-16** (13%) → **5** (6%) and **6** (5%). It should be noted that the yield for the final Wittig coupling step can be probably improved if this reaction is scaled up. Therefore, if we could duplicate the reported yield of the final coupling step of Widmer et al., our yields of **5** and **6** would improve to 12% and 11%, respectively. The overall yields for all the reported total synthesis of zeaxanthin to date are compared with our overall yields of **5** and **6** in Table 6.

Table 6. Summary of the yield of the reported synthesis of zeaxanthin.

Reported Synthesis of Zeaxanthin	Yields, number of steps		
	(±)-zeaxanthin	(*3R,3′R*)-zeaxanthin (**5**)	(*3S,3′S*)-zeaxanthin (**6**)
Loeber et al.[42], 1971. (Scheme 5, p 17)	4%, 11	—	—
Rüttimann and Mayer.[43], 1980. (Schemes 6-7, p 19-20)	—	3%, 7	3%, 7
Widmer et al.[44], 1990. (Scheme 8, p 22)	—	27%, 8	—
Soukup et al.[45], 1990 (Scheme 9, p 23)	—	39%, 11	—
Present synthesis developed in our lab	—	6%*, 8	5%*, 8

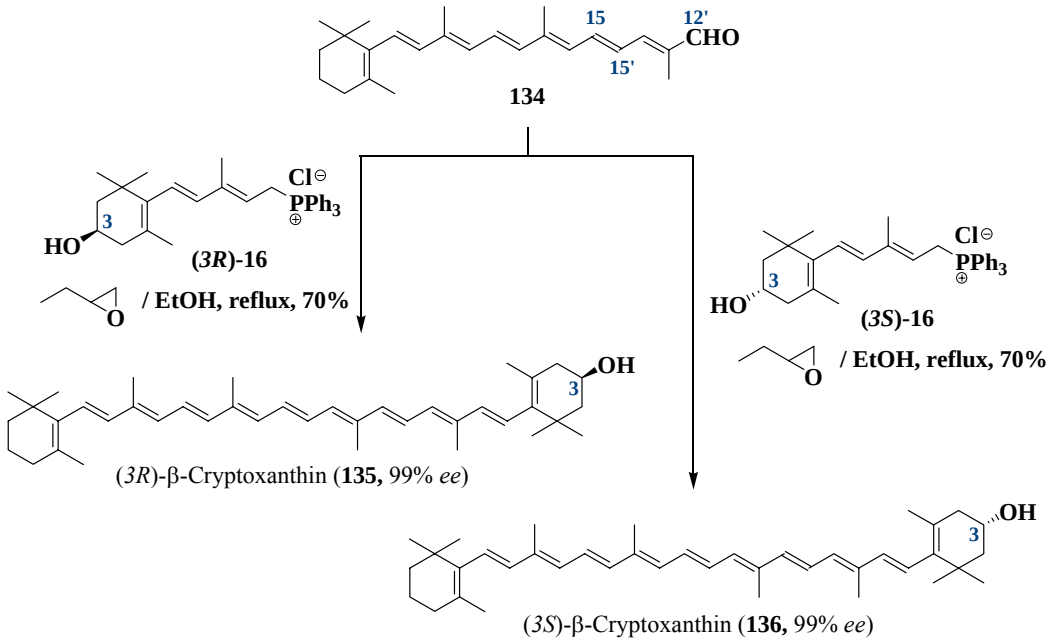
*Yields of **5** and **6** could be improved on scale-up to 12% and 11%, respectively.

With Wittig salts **(*3R*)-16** and **(*3S*)-16** at hand, we became interested in the synthesis of other carotenoids with 3-hydroxy-β-end group. One of the most

important dietary carotenoids with vitamin A activity is β-cryptoxanthin that possesses a (*3R*)-3-hydroxyl-β-end group. Therefore, we decided to investigate the application of these Wittig salts in the synthesis of (*3R*)-β-cryptoxanthin and its (*3S*)-enantiomer.

Synthesis of (*3R*)-β-Cryptoxanthin and (*3S*)-β-Cryptoxanthin

(*3R*)-β-Cryptoxanthin and its (*3S*)-enantiomer were prepared by condensation of β-apo-12'-carotenal (**134**) with Wittig salts **(*3R*)-16** and **(*3S*)-16**, respectively (Scheme 39). β-Apo-12'-carotenal (**134**) was synthesized from β-ionone according to the method of Freyschlag et al.[89]



Scheme 39. Synthesis of (*3R*)-β-cryptoxanthin (**135**) and its (*3S*)-enantiomer (**136**) from β-apo-12'-carotenal (**134**) and Wittig salts **(*3R*)-16** and **(*3S*)-16**.

The crude product of each reaction was directly subjected to low temperature crystallization from CH_2Cl_2 and hexane to afford (*3R*)-β-cryptoxanthin (**135**) and its

(*3S*)-enantiomer (**134**) in 70% isolated yield. Unfortunately, attempts to resolve enantiomeric cryptoxanthins **135** and **136** by chiral HPLC (Eluent M, Appendix I) was not successful and resulted in partial separation of these carotenoids (Figure 21). Therefore, the optical purity of **135** and **136** was assumed to be identical to that of Wittig salts **(*3R*)-16** (98% *ee*) and **(*3S*)-16** (98% *ee*). The overall yields for making Wittig salts **(*3R*)-16** and **(*3S*)-16** were 12% and 13% and as a result, the overall yield of (*3R*)-β-cryptoxanthin (**135**) and its (*3S*)-enantiomer (**134**) from (±)-α-ionone were 8% and 9%, respectively.

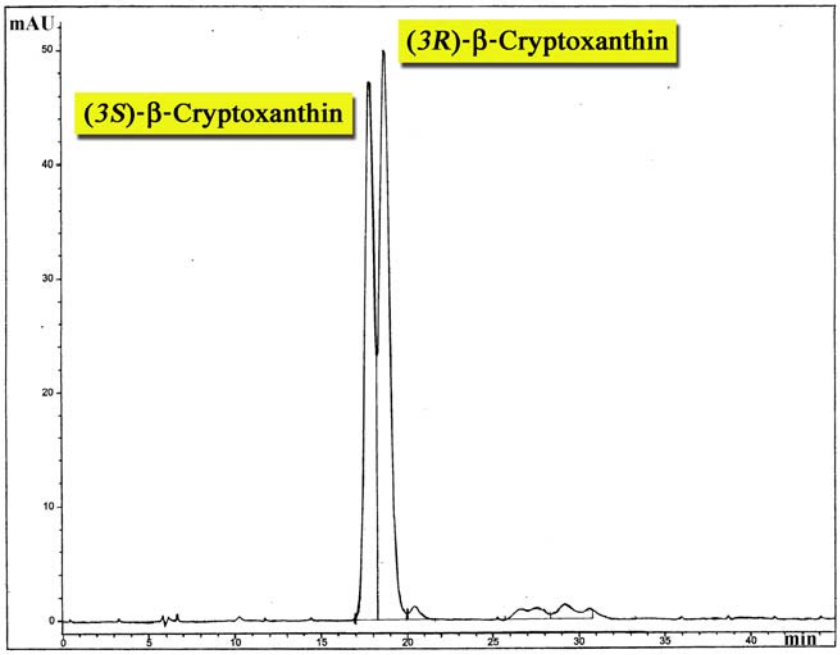


Figure 21. Chiral HPLC separation (Eluent M, Appendix I) of (*3R*)-β-cryptoxanthin (**135**) and (*3S*)-β-cryptoxanthin (**136**).

The CD spectra of (*3R*)-β-cryptoxanthin (**135**) and (*3S*)-β-cryptoxanthin (**136**) were in agreement with those of (*3R,3′R*)-zeaxanthin (**5**) and (*3S,3′S*)-zeaxanthin (**6**) that we had prepared earlier (Figure 22).

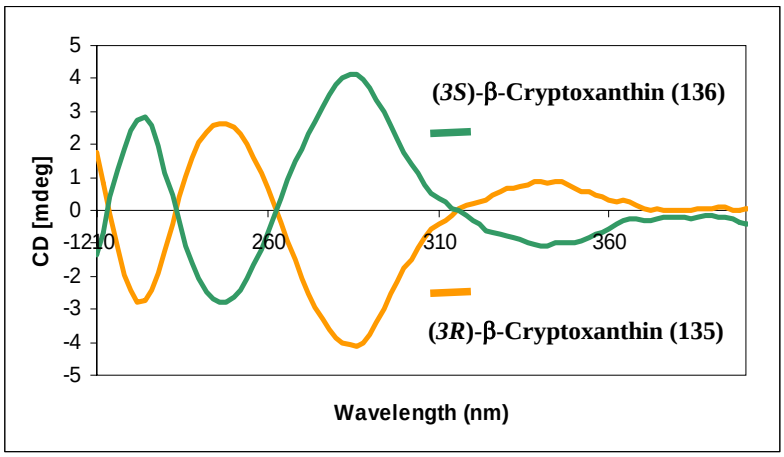
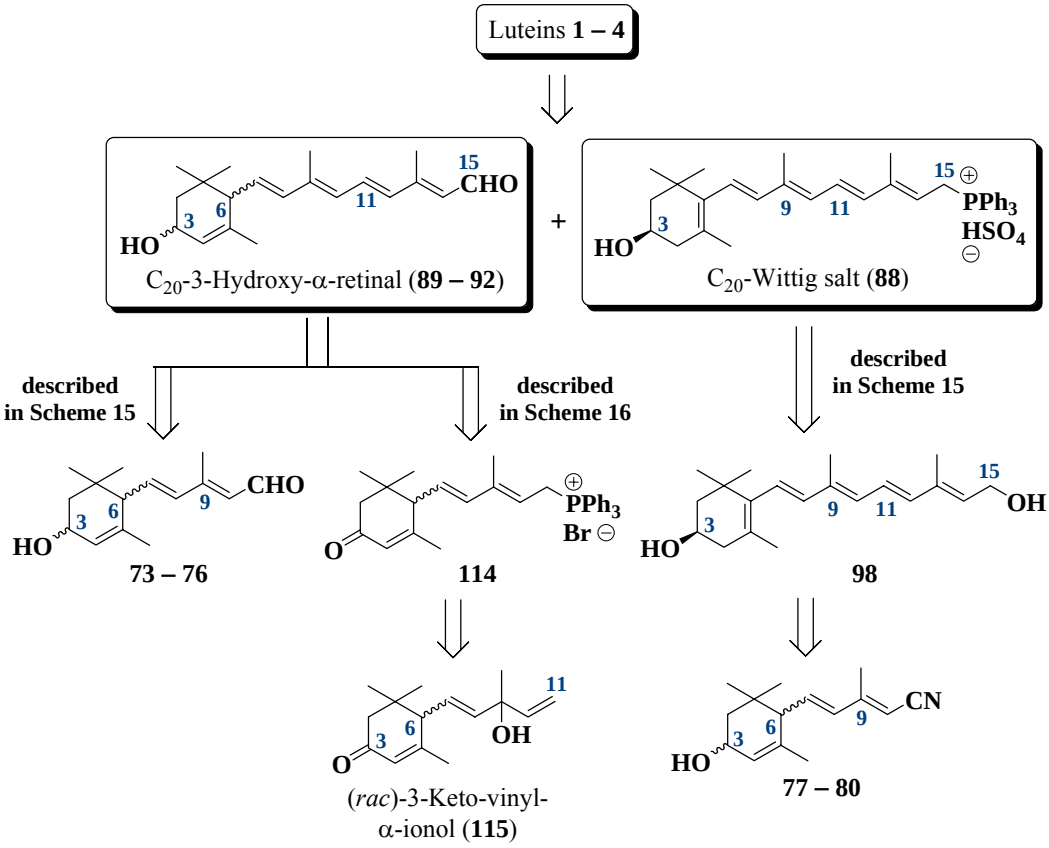


Figure 22. CD spectra of (*3R*)-β-cryptoxanthin (**135**) and (*3S*)-β-cryptoxanthin (**136**) in hexane:ether:methanol = 10:3:1.

Total Synthesis of Luteins 1 – 4 Using C_{20}+C_{20} Coupling Strategy

As described earlier, an alternative approach to the synthesis of luteins **1** – **4** is by employing C_{20}-aldehyde + C_{20}-Wittig salt building blocks that can be joined by a Wittig coupling reaction to form this C_{40}-carotenoid and its stereoisomers (Scheme 40). This strategy takes advantage of the fact that any amount of *15Z*-isomer of lutein that is formed as a result of this coupling can be transformed into the thermodynamically more stable *all-E*-isomer. We investigated two routes for the synthesis of C_{20}-aldehydes, 3-hydroxy-α-retinals (**89** – **92**). The first route involved elongation of C_{15}-hydroxyaldehydes **73** – **76** that we had prepared previously. The second route was inspired by the relative ease with which the Wittig salt **114** had been synthesized from (±)-α-ionone *via* (±)-3-keto-vinyl-α-ionol (**115**) by Widmer et al. The C_{20}-Wittig salt (**88**) could be obtained from the double bond isomerization of C_{15}-hydroxynitriles **77** – **80** followed by reduction with DIBAL-H and elongation to C_{20}-diols **98**.

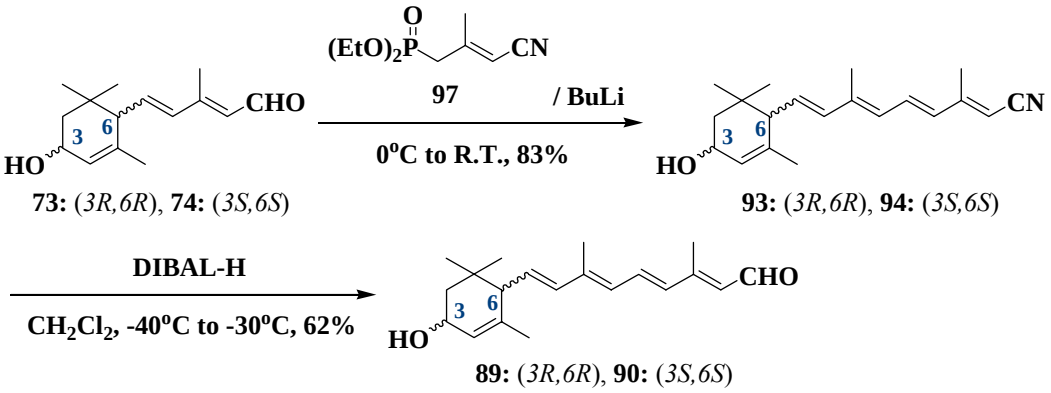


Scheme 40. Retrosynthetic summary of the synthesis of lutein employing C_{20}+C_{20} coupling strategy.

Synthesis of C_{20}-3-Hydroxy-α-retinals (89 – 92) from Hydroxyaldehydes 73 – 76

To arrive at the optically pure C_{20}-hydroxyaldehydes **89** – **92**, we planned to employ enzyme-mediated acylation of **89** – **92** or its precursors, hydroxyaldehydes **73** – **76**. While hydroxyaldehydes **73** – **76** were available in high optical purity from our previous synthesis, we decided to use the racemic mixture of *trans*-C_{15}-hydroxyaldehydes **73** and **74** to first examine the efficacy of this C_{20}+C_{20} coupling strategy. The racemic mixture of C_{15}-hydroxyaldehydes **73** and **74** was elongated with C_5 synthon **97** to afford (±)-C_{20}-hydroxynitriles **93** and **94** in 83% isolated yield after

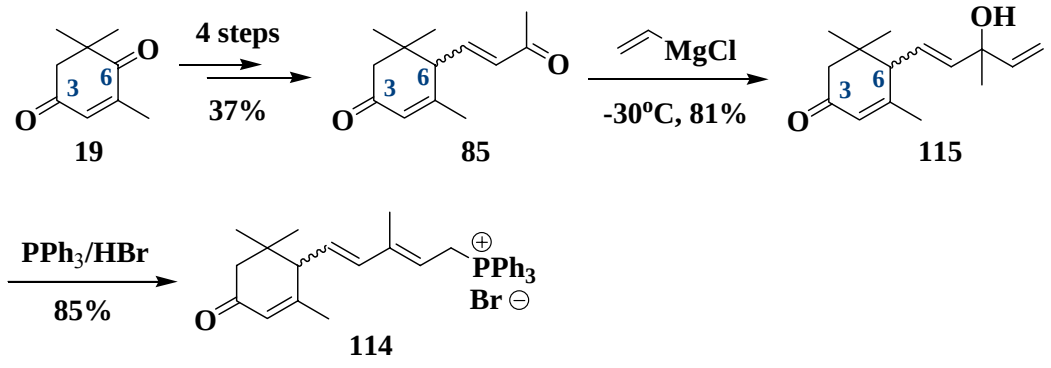
column chromatography (Scheme 41). DIBAL-H reduction of these hydroxynitriles gave C_{20}-hydroxyaldehydes **89** and **90** in 62 % yield after chromatography.



Scheme 41. The synthesis of (±)-C_{20}-hydroxyaldehydes **89** and **90** from (±)-C_{15}-hydroxyaldehydes **73** and **74**.

Synthesis of C_{20}-3-Hydroxy-α-Retinals (89 – 92) from (±)-α-Ionone *via* (±)-3-Keto-Vinyl-α-Ionol (115)

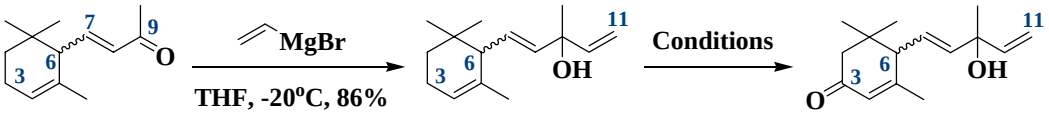
The key starting material for the synthesis of C_{20}-hydroxyaldehydes **89** – **92** by this alternative route was Wittig salt **114** that has been previously prepared by Widmer et al. in 25% overall yield as according to Scheme 42.[90]



Scheme 42. Synthesis of Wittig salt **114** according to the method of Widmer et al.[90]

We prepared the ketoionol **115** by vinylation of α-ionone with vinylmagnesium

bromide according to a published procedure[37] in 86% isolated yield followed by allylic oxidation (Scheme 43).



(*rac*)-α-Ionone

(*rac*)-Vinyl-α-ionol (**116**)

(*rac*)-3-Keto-vinyl-α-ionol (**115**)

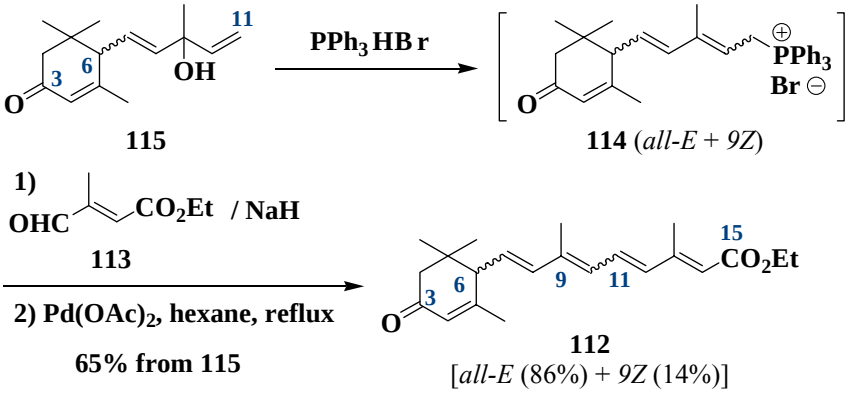
Scheme 43. Preparation of (±)-3-keto-α-ionol (**115**). Conditions are described in Table 7.

In the following step, **116** was converted to **115** by bleach oxidation in 79% isolated yield after chromatography.[59] Ketone **115** was also prepared from allylic oxidation of **116** in 60% yield using dirhodium(II) caprolactamate as catalyst.[58] This oxidation could also be accomplished in 45% yield by the method of Yu and Corey using Pd(0) as catalyst.[57] The results of these oxidation reactions are summarized in Table7.

Table 7. Allylic oxidation of vinyl-α-ionol (**116**) to 3-keto-vinyl-α-ionol (**115**).

Entry	Conditions	Isolated Yield (%)
1	Household bleach (5.25% NaOCl), 70% TBHP in H_2O, K_2CO_3, EtOAc, -2 to 5˚C, 17 h	79
2	$Rh_2(cap)_4$, 5.5M TBHP in decane, K_2CO_3, CH_2Cl_2, R.T., 50 h	60
3	Pd/C, 5.5M TBHP in decane, K_2CO_3, Hexane, 0˚C to R.T., 50 h	45

Using conditions developed by Widmer et al.[90], we transformed ketone **115** into the C_{15}-Wittig salt **114**. C_{15}-Wittig salts of carotenoids are usually crystallized to remove the 9*Z*-isomers that are not crystallized well. However, we were unable to crystallize **114** and this Wittig salt was directly used in the following step as a mixture of *all-E* and 9*Z* isomers. Elongation of **114** with (*E*)-ethyl-3-formyl-2-butenoate (**113**) afforded the C_{20}-ketoester **112** as a mixture of *all-E*, 9*Z* and 11*Z*-isomers in 65% isolated yield from **115** (Scheme 44).

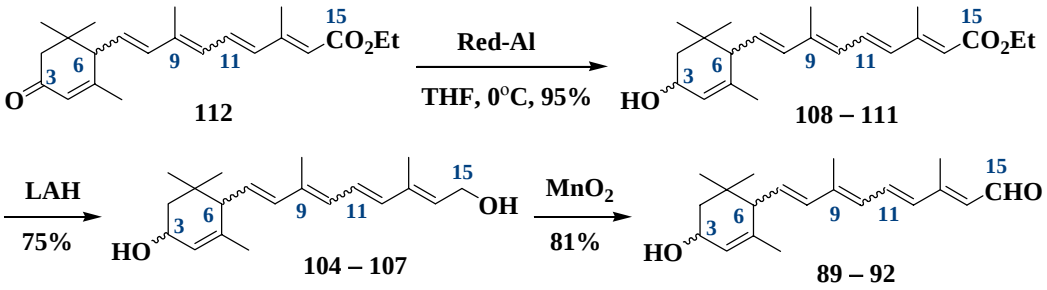


Scheme 44. Preparation of C_{20}-3-ketoester **112** from **115**.

It should be noted that aldehyde **113** was prepared as an *all-E* isomer according to a known method.[91] Fortunately, the 11*Z* isomers of carotenoids are sterically hindered and are readily converted to their *all-E* isomers.[46] Thus the 11*Z* bond in ketoester **112** was isomerized with $Pd(OAc)_2$ in refluxing hexane to yield a mixture of *all-E* (86%) and 9*Z* (14%) isomers of **112**. A small sample of this isomeric mixture was separated by semipreparative HPLC and the individual isomers were fully characterized. Although **112** appears to be a useful precursor for the synthesis of C_{40} carotenoids, the preparation of this ketoester has not been previously reported.

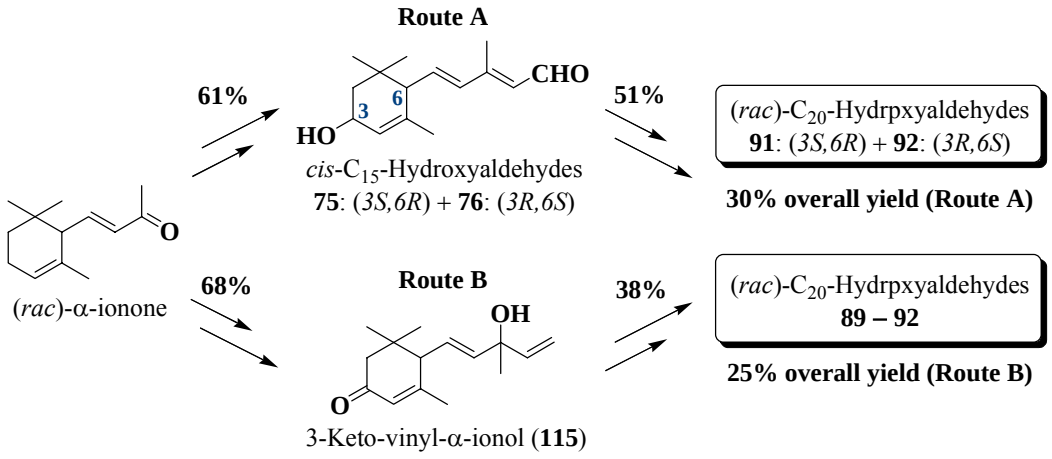
Reduction of **112** (*all-E+9Z*) with $NaAlH_2(OCH_2CH_2OMe)_2$ (Red-Al) gave C_{20}-

hydroxyesters **108** – **111** (*all-E+9Z*) in nearly quantitative yield which was then further reduced with LAH to afford C_{20}-diol **104** – **107** as a mixture of *all-E+9Z* in 75% yield (Scheme 45). Due to the unstable nature of this compound, the diol **104** – **107** was immediately used in the following step. The oxidation of diol **104** – **107** with MnO_2 gave C_{20}-hydroxyaldehyde **89** – **92** (*all-E+9Z*) in 81% isolated yield after chromatography.



Scheme 45. Preparation of C_{20}-hydroxyaldehyde **89** – **92** from C_{20}-ketoester **112**.

In summary, two routes were developed for the synthesis of C_{20}-hydroxyaldehydes **89** – **92** from (±)-α-ionone and the yields are compared in Scheme 46.



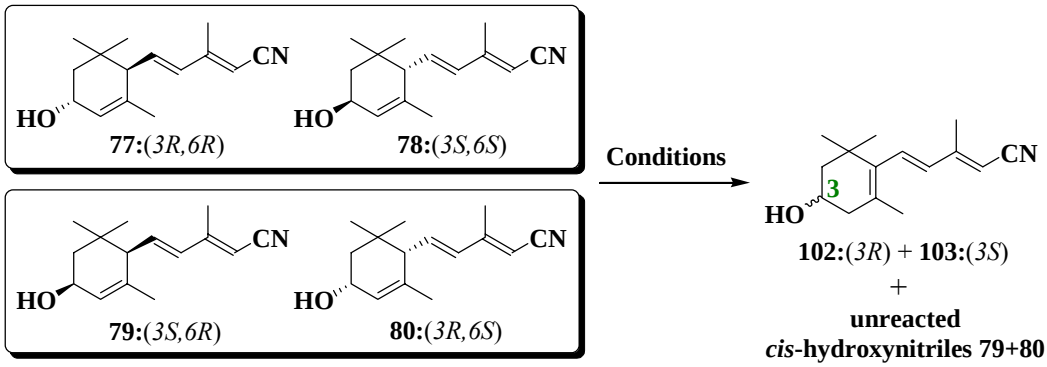
Scheme 46. The overall yield summary for the preparation of C_{20}-hydroxyaldehydes **89** – **92** according to routes A and B.

According to the first route (Route A), the (*cis*)-C_{20}-hydroxyaldehydes **91** and **92** were obtained in 51% from (*cis*)-C_{15}-hydroxyaldehydes **75** and **76**. As described earlier in the synthesis of luteins **1** – **4**, aldehydes **75** and **76** were prepared from (±)-α-ionone in 61% yield. Therefore, the overall yield for preparing the racemic mixture of **91** and **92** according to route A was 30%. In the second route (Route B), the racemic mixture of C_{20}-hydroxyaldehydes **89** – **92** were obtained in 25% overall yield from (±)-α-ionone *via* 3-keto-vinyl-α-ionol (**115**); details of this synthesis was described earlier in Scheme 43. Although the overall yield of (±)-C_{20}-hydroxyaldehydes by routes A and B were comparable, route A was more appealing because we had previously prepared the four optical isomers of C_{15}-hydroxyaldehydes **73** – **76** in a high *ee*. However, with Route B, it was not clear to us whether the separation of various stereoisomers of (±)-C_{20}-hydroxyaldehydes **89** – **92** could be accomplished.

Synthesis of C_{20}-Wittig Salt 88

Based on our previous experience with the double bond isomerization of 3-hydroxy-α-ionone (**120** – **123**) to 3-hydroxy-β-ionone (**42**), we explored the base-promoted double bond isomrization of 3-hydroxy-α-ionylideneacetonitrile (**77** – **80**) to 3-hydroxy-β-ionylideneacetonitrile (**102** – **103**) (Table 8). This is because nitriles **77** – **80** were readily available from (±)-α-ionone from our earlier synthesis of luteins and **102** and **103** could serve as precursors to C_{20}-Wittig Salt **88** as shown in Scheme 40. Initially, we examined this reaction with a racemic mixture of hydroxynitriles **77** – **80** employing KOH or KF-alumina as base.

Table 8. Base-promoted double bond isomerization of hydroxynitriles **77** – **80** to hydroxynitriles **102** – **103**.



Entry	Conditions	Conversion to **102**+**103** (%)	Unreacted (%)*, **77**+**78** : **79**+**80**
1	KOH (0.7 eq), EtOH, reflux, 24 h	N.R.	100, 1.0 : 1.0
2	KOH (0.7 eq), n-PrOH, 90 °C, 15 h	10	90, 1.0 : 1.6
3	KOH (17 eq), $MeOCH_2CH_2OH$, 120 °C, 24 h	65	35, 1.0 : 3.0
4	KOH (1.7 eq), $HOCH_2CH_2OH$, 120 °C, 24 h	61	39, 1.0 : 2.3
5	KF-Alumina (40% by wt, 2.3 eq), DMF, 120 °C, 29 h	64	36, 1.0 : 4.4
6	KF-Alumina (40% by wt, 1.4 eq), $HOCH_2CH_2OH$, 120 °C, 16 h	50	50, 1.0 : 2.2

*Ratios were determined by HPLC (Eluent A, Appendix I). N.R. = No reaction.

The reactions with KOH in different alcohols revealed that *trans*-hydroxynitriles **77**+**78** began to slowly isomerize to the desired product **102**+**103** at about 120 °C. Meanwhile, the double bond isomerization of the *cis*-hydroxynitriles **79**+**80** was considerably slower at this temperature. Despite the fact that the reaction with KOH was allowed to proceed for extended periods of time, no greater than 64% of **77** – **80** could be converted to **102**+**103**. Unfortunately, prolonged heating at high temperature in the presence of KOH resulted in considerable degradation of product and gave a poor yield of about 30%.

Following these disappointing results, we decided to promote this isomerization with a milder base such as KF-alumina to avoid the degradation of product. The utility of alumina coated with KF as a versatile solid-supported base for double bond isomerization of olefins to the thermodynamically more stable form has been previously documented.[92] This reagent has also been used in Aldol condensation[93], and Michael addition reaction of chalcones and malononitriles.[94] The isomerization of hydroxynitriles **77** – **80** with KF-alumina in DMF after 29 h at 120 °C resulted in 64% conversion of these nitriles and afforded **102**+**103** in 40% yield. In addition, only 6% of *trans*-hydroxynitriles **77**+**78** and 30% of *cis*-hydroxynitriles **79**+**80** remained unreacted.

To better understand the reason for the much slower conversion of *cis*-hydroxynitriles **79**+**80** to **102**+**103** in comparison with *trans*-hydroxynitriles **77**+**78**, conformational analyses of these hydroxynitriles was carried out using the Spartan MMFF method. As shown in Figure 23, the *cis*-hydroxynitriles **79**+**80** can exist in two half-chair conformations A and B with the calculated energies of 28.37 and 25.04 Kcal/mol, respectively. On the contrary, the preferred half-chair conformation of *trans*-hydroxynitriles **77**+**78** has the energy of 27.43 Kcal/mol. While the higher energy of *trans*-hydroxynitriles (**77**+**78**, 27.43 Kcal/mol) relative to the energy of conformation B of the *cis*-hydroxynitriles (**79**+**80**, 25.04 Kcal/mol) is unexpected, it may explain the higher reactivity of the former relative to the latter in our isomerization reactions.

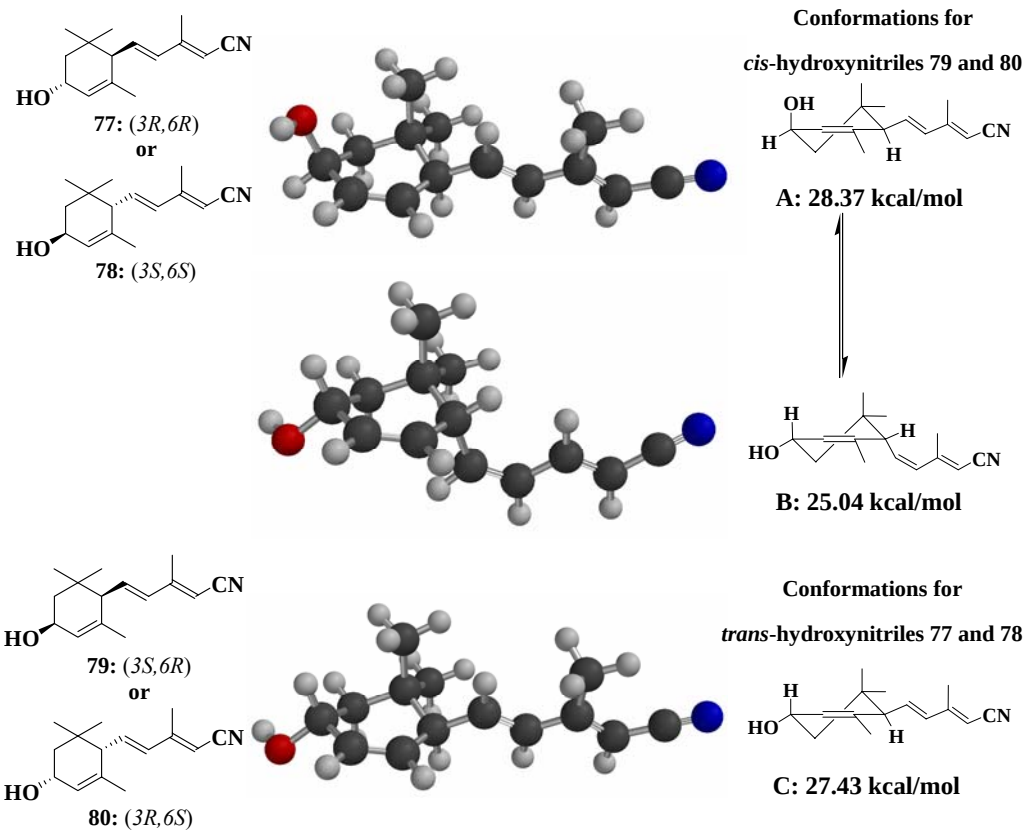
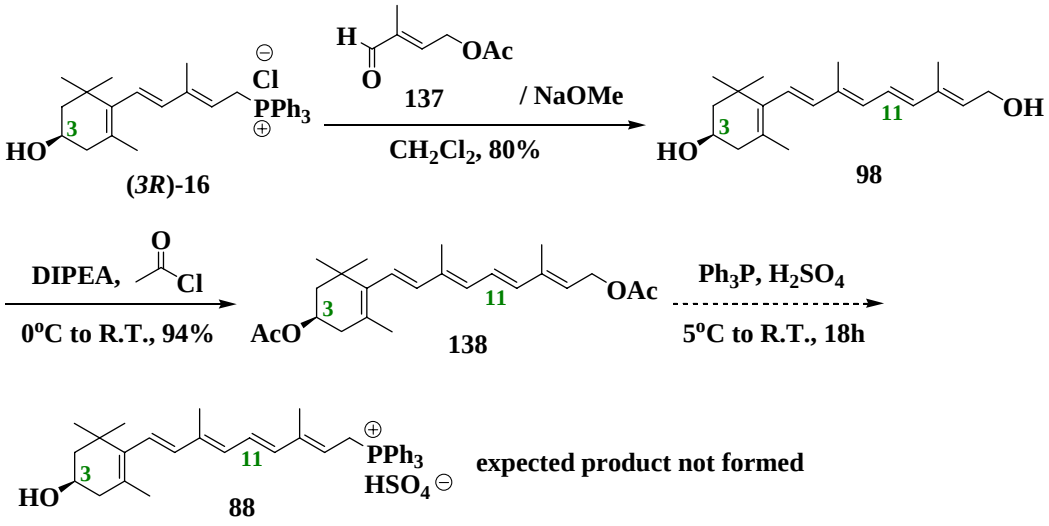


Figure 23. The half-chair conformations of *cis*-hydroxynitriles **79**+**80** and *trans*-hydroxynitriles **77**+**78** and their corresponding energies calculated by Spartan MMFF method.

Aside from the relatively low yields, the major drawback with the base-promoted double bond isomerization reactions of hydroxynitriles **77** – **80** was separation of the unreacted nitriles from hydroxynitriles **102**+**103** that could only be accomplished by repeated column chromatography. Therefore, in order to continue with the synthesis of C_{20}-Wittig salt **88**, the Wittig salt **(*3R*)-16** that we had previously prepared from 3-hydroxy-β-ionone (see Scheme 37) was elongated with C_5-synthon **137** to afford C_{20}-diol **98** in 80% isolated yield after chromatography (Scheme 47).



Scheme 47. Synthesis of C_{20}-Wittig salt **88**. DIPEA = *N,N*-diisopopylethylamine.

After protection of the C_{20}-diol **98** with acetyl chloride, the diacetate **138** was treated with Ph_3P and H_2SO_4 to form 3-hydroxy-retinaltriphenylphosphonium sulfate (**88**). However, this reaction did not produce any Witig salt and the diacetate **138** was partially degraded. As mentioned earlier in Scheme 14, retinaltriphenylphosphonium salts **86**[52-54] have been successfully prepared for the synthesis of β,β-carotene but the acylated or hydroxylated derivatives of this Wittig salt **88** have not been reported to date. We also tried to prepare the chloride salt but this reaction also failed to produce any product.

In conclusion, our feasibility studies for construction of the optically inactive luteins by a C_{20}+C_{20} Wittig coupling strategy indicated that the C_{20}-3-hydroxy-α-retinals (**89** – **92**) could be prepared by two different routes in overall yields of 25% and 30%. However, the preparation of the C_{20}-Wittig salt proved to be problematic and it is unclear to us whether conditions for making this salt can be worked out.

Considering that the overall yield for the synthesis of the diacetate **138** from (±)-α-ionone via-3-hydroxy-β-ionone was about 11%, any procedure that could provide the Wittig salt **88** would have to be highly efficient for this C_{20}+C_{20} synthetic strategy to be viable. Therefore at present, the C_{15}+C_{10}+C_{15} coupling strategy for the synthesis of lutein and its stereoisomers is the most practical route to these carotenoids.

CONCLUSION

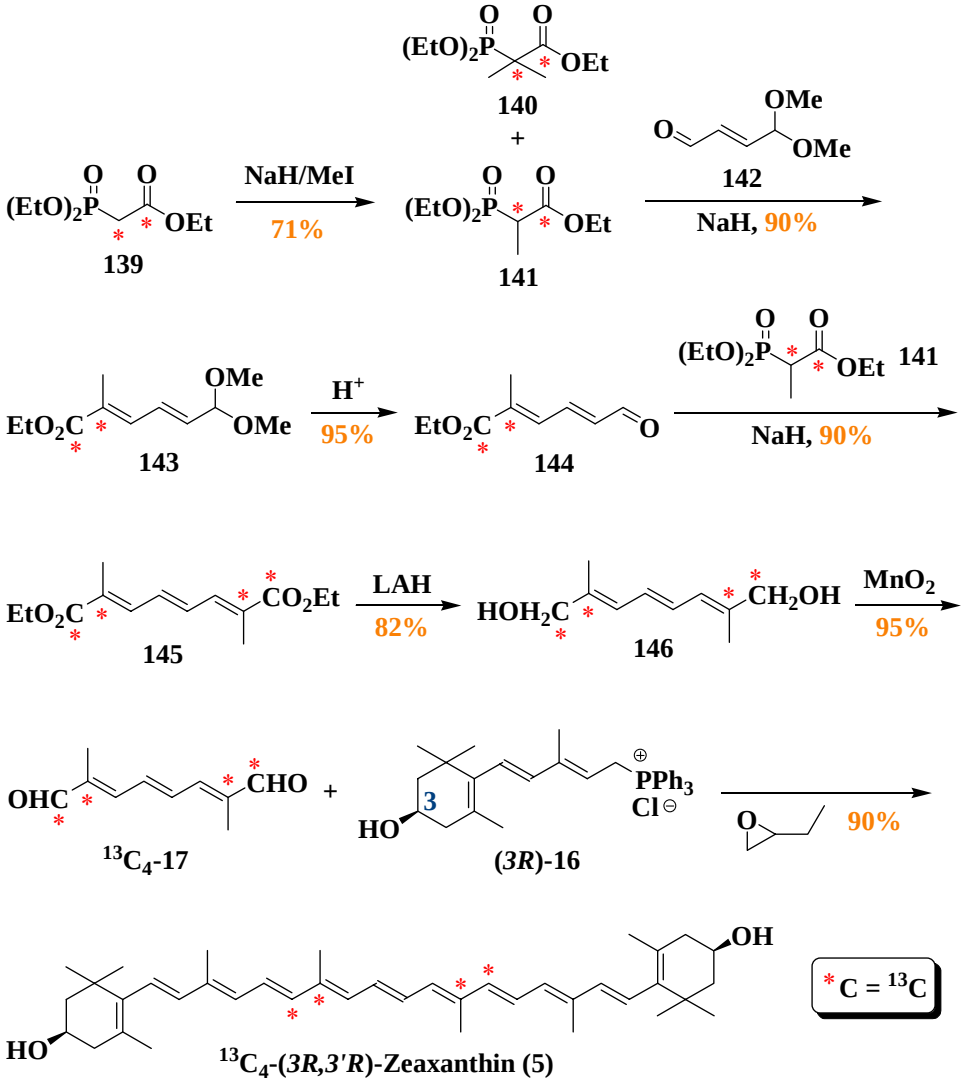
We have developed methodologies for the synthesis of lutein, zeaxanthin, β-cryptoxanthin, and their stereoisomers from (±)-α-ionone employing a C_{15}+C_{10}+C_{15} coupling strategy. Dietary (*3R,3′R,6′R*)-lutein (**1**, 5%, 98% *ee*) and three of its stereoisomers (*3R,3′S,6′S*)-lutein (**2**, 6%, 98% *ee*), (*3R,3′S,6′R*)-lutein (**3**, 5%, 91% *ee*), and (*3R,3′R,6′S*)-lutein (**4**, 6%, 94% *ee*) were each prepared from (±)-α-ionone *via* optically pure C_{15}-hydroxyaldehydes **73** – **76** that served as key intermediates in this synthesis. This methodology can also be applied to the synthesis of the other four stereoisomers of luteins in which the configuration at C3 position is *S* while the stereochemistry at C3' and C6' remains the same as luteins **1** – **4**.

Employing a similar coupling strategy, the synthesis of (*3R,3′R*)-zeaxanthin (**5**, 6%, 98% *ee*), (*3S,3′S*)-zeaxanthin (**6**, 5%, 98% *ee*), (*3R*)-β-cryptoxanthin (**135**, 8%, 99% *ee*), and (*3S*)-β-cryptoxanthin (**136**, 9%, 99% *ee*) was accomplished from C_{15}-Wittig salt **16**. This Wittig salt was prepared as *3R* and *3S* isomers in high enantiomeric purity from (±)-α-ionone *via* (*3R*)- and (*3S*)-3-hydroxy-β-ionone (**42**).

As mentioned earlier, the main objective of the research described in this dissertation was to develop methodologies that could be applied to the synthesis of

^{13}C-labeled lutein and its stereoisomers for metabolic studies. This can now be accomplished since the C_{15}-hydroxyaldehydes **73** – **76** and the C_{15}-Wittig salts **(*3R*)-16** and **(*3S*)-16** that are the required end-groups for the synthesis of lutein and its stereoisomers have been prepared in high optical purity. However, because of the lengthy nature of the synthetic steps and the low overall yields of these end-groups, the ^{13}C-labels will have to be incorporated into the protected C_{10}-Wittig salt **72**.

This approach has been successfully applied to the synthesis of $^{13}C_4$-(*3R,3'R*)-zeaxanthin by Khachik et al. as shown in Scheme 48.[95]

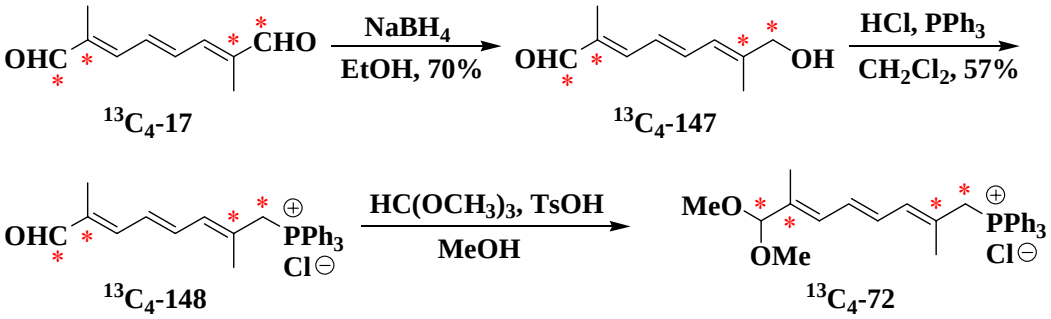


Scheme 48. Synthesis of $^{13}C_4$-(*3R,3'R*)-zeaxanthin (**5**) by Khachik et al.[95]

all-E-2,7-Dimethylocta-2,4,6-triene-1,8-dial (C_{10}-dialdehyde, **17)** labeled with four ^{13}C was synthesized from commercially available $^{13}C_2$-triethyl phophonoacetate **139** and fumarylaldehyde dimethylacetal in six steps in an overall yield of 43-46%. As mentioned earlier, C_{10}-dialdehyde **17** is a common building block for the central polyene chain of many carotenoids and has been used in the commercial synthesis of zeaxanthin.[38] $^{13}C_2$-Triethyl 2-phosphonopropionate **141** was prepared from **139** by alkylation with methyl iodide that also afforded **140** (18%) as a side product. According to this procedure, the unreacted **139** was recovered in the purification process. The use of inexpensive ^{13}C-labelled methyl iodide in alkylation step could also allow the introduction of two additional ^{13}C labels into the molecule. Because of the polymerization nature of fumarylaldehyde, fumarylaldehyde dimethylacetal was catalytically hydrolyzed to fumarylaldehyde monomethylacetal (**142**); the WHE reaction between **141** and this monoacetal resulted in the formation of $^{13}C_2$-6,6-dimethoxy-2-methyl-*E,E*-2,4-hexadienoate (**143**). Compound **143** was then deprotected to **144** and elongated with **141** to yield $^{13}C_4$- (*all-E*)-2,7-dimethylocta-2,4,6-triene-1,8-diacid ethylester **145**. Reduction of **145** by LAH followed by oxidation of the resulting diol **146** with MnO_2 resulted the formation of the desired $^{13}C_4$-C_{10}-dialdehyde **17**. In the final step of the synthesis, $^{13}C_4$-(*3R,3'R*)-zeaxanthin (**5**) was then obtained by double Wittig coupling of $^{13}C_4$-C_{10}-dialdehyde **17** and the Wittig salt (*3R*)-**16** to afforded $^{13}C_4$- (*3R,3'R*)-zeaxanthin (**5**) in 90% yield. Therefore, employing this strategy, Khachik et al. incorporated four ^{13}C-labels into (*3R,3'R*)-zeaxanthin at positions 12, 13, 12′, and 13′.

The protected C_{10}-Wittig salt **72** that was used in our synthesis of luteins **1** – **4**

has been previously prepared from C_{10}-dialdehyde **17** by Bernhard et al.[47] Therefore, $^{13}C_4$-C_{10}-dialdehyde **17** can be prepared by the method of Khachik et al. and subsequently transformed into the protected C_{10}-Wittig salt **72** by the method of Bernhard et al. (Scheme 49).



Scheme 49. Synthesis of protected C_{10}-Wittig salt **72** according to the method of Bernhard et al.[47]

Considering that C_{10}-dialdehyde **17** labeled with 4 to 6 carbon-13 can be prepared in gram quantities from $^{13}C_2$-triethyl phophonoacetate **139** and $^{13}CH_3I$, the synthesis of ^{13}C-labeled lutein and its stereoisomers can be accomplished in relatively large quantities for metabolic studies.

EXPERIMENTAL SECTION

All experiments were carried out in an anhydrous nitrogen atmosphere; reaction vessels were dried in the oven prior to use. All solvents were dried over molecular sieves (4 Å) or directly used from an unopened bottle. Saturated solutions of $NaHCO_3$ and NH_4Cl refer to saturated solutions of the salt in water. Brine refers to a saturated solution of NaCl in water.

Reactions were monitored by using thin-layer chromatography (TLC) on Merck silica-coated glass plates treated with UV-active binder, with compounds being identified in one or more of the following methods: UV (254 nm) or phosphomolybic acid indicator.

Reactions were also monitored by HPLC employing normal phase, reversed phase, and chiral-columns under various conditions as described below. The analyses were performed on an HPLC system (model 1100; Agilent Technology, CA) equipped with a quaternary solvent delivery system (model G1311A), an autosampler (model G1313A), a thermostat-controlled column compartment (model G1316A), and a photodiode array detector (model G1315B). The data were stored and processed by a computer (Dell with Windows 2000 using HP Chem-Station software.

Normal phase HPLC separations were carried on a silica-based nitrile bonded column (25 cm length x 4.6 mm i.d.; 5-μm spherical particle; part # PSS830915 Waters Corporation, MA. The column flow rate was 0.7 mL/min.

C_{18}-reversed phase HPLC separations were carried out on a C_{18}-Microsorb column (25 cm length x 4.6 mm i.d.; 5-μm spherical particle; Varian Instrument Co., MA). Pump A pumped a mixture of acetonitrile (90%) and methanol (10%) and pump

B pumped a mixture of hexane (45%), methylene chloride (45%), methanol (10%). The column flow rate was 0.7 mL/min

All semipreparative normal phase HPLC separations were carried out on a silica-based nitrile bonded column (25 cm length x 10 mm i.d.; 10-μm spherical particle; Waters Corporation, MA). Various mobile phases were used with this column at a flow rate of 3mL/min.

All chiral HPLC separations were carried out on an amylose tris-(3,5-dimethylphenylcarbamate) chiral analytical column (Chiralpak AD, 25 cm length x 4.6 mm i.d.), protected with a silica gel guard cartridge (3 cm length x 4.6 mm i.d.; 5-μm particle); flow rate = 0.7 mL/min.

All operations and HPLC analyses were conducted under yellow laboratory light to prevent photo-isomerization and degradation of carotenoids and their precursors. ^{1}H NMR spectra were recorded on a Bruker DRX-400 MHz spectrometer with $CDCl_3$ (7.27 ppm) as internal standard. ^{1}H noise-decoupled ^{13}C spectra were recorded on a Bruker DRX-400 MHz at 100 MHz with chloroform (77.0 ppm) as an internal standard. Low resolution (LRMS) and high resolution (HRMS) mass spectra were obtained on a JEOL AccuTOF CS mass spectrometer (ion source: ESI, EI, CI or DEI; needle voltage: 2300 v; flow rate: 50 μl/min; desolvation chamber temperature = 250˚C; data acquisition time: 2 min). Circular dichroism (CD) was carried out on a JASCO (Model J810) instrument. A mixture of hexane, ether, and methanol (10:3:1) was used as the background solvent. All compounds were determined to be >95% pure by ^{1}H NMR or HPLC analysis, unless otherwise noted. All new compounds were fully characterized using ^{1}H NMR, ^{13}C NMR, UV/Vis, CD, and low resolution and

high resolution mass spectrometry.

Palladium(II)-Mediated Oxidation of (±)-α-Ionone to (±)-3-Keto-α-Ionone (85) (**Table 1, Entry 1**)**.** Freshly distilled (±)-α-ionone (1.00 g, 5.20 mmol) in dichloromethane (10 mL) was cooled down in an ice-salt bath to 0°C under N_2 and was treated with K_2CO_3 (0.180 g, 1.30 mmol) and Pd/C (10 wt.% on C, 0.150 g ~ 15 mg Pd, 0.14 mmol). A 5.5 M anhydrous solution of TBHP in decane (5.0 mL, 28 mmol) was added to the mixture at 0°C. The mixture was stirred for 24 h at 0°C and 12 h at room temperature (R.T.) under N_2. The solids were removed by filtration through celite and the filtrate was washed with water (3 X 20 mL), brine, and dried over Na_2SO_4. The solvent was removed under reduced pressure to give 1.3 g of a yellow oil. The crude product was purified by column chromatography (silica gel, hexane:acetone, from 98:2 to 92:8, R_f = 0.53 in hexane:acetone = 7:3) followed by crystallization from ether:hexane (1.4:1) to yield (±)-3-keto-α-ionone (**85**) as a white solid: 0.57 g, 2.8 mmol, 53%, mp 75-76°C. ^{1}H NMR (400MHz, $CDCl_3$) δ 0.88 (s, 3H), 0.95 (s, 3H), 1.78 (s, 3H), 2.00 (d, J = 16.8, 1H), 2.15 (s, 3H), 2.24 (d, J = 16.8, 1H) 2.61 (d, J = 9.5, 1H), 5.83 (s, 1H), 6.06 (d, J = 15.8, 1H), 6.57 (dd, J = 9.5, 15.8, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 23.3, 27.1, 27.4, 27.7, 36.5, 47.1, 55.2,126.7, 133.6, 143.4, 159.0, 197.4, 198.1. MS (ESI^+) calculated for $C_{13}H_{18}O_2$ $[M+H]^+$ 207.1385, found 207.1232.

$Rh_2(cap)_4$ Catalyzed Oxidation of (±)-α-Ionone to (±)-3-Keto-α-Ionone (85) (Table 1, Entry 2). To a solution of (±)-α-ionone (1 g, 5.2 mmol) in 10 mL of CH_2Cl_2

was added K_2CO_3 (0.36 g, 2.6 mmol), and dirhodium(II) caprolactamate [$Rh_2(cap)_4$] (2.1 mg, 0.005 mmol), followed by 6.3 M *tert*-butylhydroperoxide (4.1 mL, 26 mmol). The reaction mixture was stirred at R.T. for 22 h and then filtered through celite. The product was washed with H_2O (2 × 100 mL), the organic layer was dried over Na_2SO_4, and concentrated *in vacuo* to give a pale yellow oil. Purification by column chromatography (silica gel, hexane:acetone, from 98:2 to 92:8) afforded 0.8 g of a colorless oil which was subjected to crystallization (ether:hexane=1.4:1) to obtain white solids of (±)-3-keto-α-ionone (**85**) (0.61 g, 63%). The NMR data were shown to be identical with **85** reported previously.

Bleach Oxidation of (±)-α-Ionone to (±)-3-Keto-α-Ionone (85) (Table 1, Entry 3). Freshly distilled (±)-α-ionone (20 g, 0.10 mol) was transferred into a 500 mL three-necked flask using EtOAc (103 mL, 92.1 g, 1.05 mol). K_2CO_3 (1.44 g, 10.4 mmol) was added and the mixture was cooled down in an ice-salt bath to 0°C under N_2. A 70% solution of TBHP in water (89 mL, 70% of 80 g ≈ 56 g, 0.62 mol) was added dropwise to the mixture under N_2 at 0°C in 30 min. Household bleach containing 5.25% NaOCl (295 g, 15.5 g NaOCl, 0.208 mol) was then added over a period of 5 h at -5 to 0°C. After the addition was completed, the reaction mixture was stirred at 0°C for an additional hour. The organic layer was removed and the water layer was washed with EtOAc (2 X 100 mL). The combined organic layer was washed with water (2 X 150 mL), dried over Na_2SO_4, and evaporated to give 26.8 g of a yellow oil. The crude product was purified by column chromatography (silica gel, hexane:acetone, from 98:2 to 92:8) followed by crystallization (ether:hexane=1.4:1)

to yield (±)-3-keto-α-ionone (**85**) (13.7 g, 66.4 mmol, 64%) as a white solid. The NMR data of this white solid were shown to be identical with **85** reported previously.

Synthesis of (7*E*,9*E*)-3-Keto-α-Ionylideneacetonitrile (81) and (7*E*,9*Z*)-3-Keto-α-Ionylideneacetonitrile (82) from (±)-3-Keto-α-Ionone (85). Sodium hydride (0.43 g of 60% suspension in oil ≈ 0.26 g, 10 mmol) was placed in a three-necked flask equipped with a nitrogen inlet and a thermometer and washed with hexane (2 X 10 mL). TBME (30 ml) was added and the mixture was cooled to 0°C. Diethyl cyanomethylphosphonate (0.96 g of 98% pure, 0.95 g, 5.3 mmol) in 10 mL TBME was added to the suspension at 5-10˚C under N_2 and the mixture was allowed to stir at R.T. for 1 h. The reaction mixture was cooled down in an ice bath and (±)-3-keto-ionone (1.0 g, 4.9 mmol) in 10 mL TBME was added dropwise in 30 min at 0-5°C. After stirring for 6 hours at R.T., the reaction was quenched with water and the organic layer was removed. The aqueous layer was extracted with TBME (2 X 20 mL). The combined organic layer was washed with water, dried over Na_2SO_4, and evaporated to dryness. The crude product (1.1 g) was purified by column chromatography (hexane:acetone, from 98:2 to 95:5) to yield a mixture of **81** and **82** (0.9 g, 4 mmol, 81%) as a pale yellow oil. The product was shown by analytical normal phase HPLC (Eluent A, Appendix I) as well as ^{1}H- and ^{13}C-NMR to consist of **81** (75%) and **82** (25%) [ratio of isomeric mixture: *7E,9E*:*7E,9Z* = 3:1]. Crystallization from ethanol at -20°C gave the (*7E,9E*)-isomer (**81**) as a white crystal (0.45 g, 1.9 mmol, 40%), mp 93-95°C. Analytically pure samples of **82** were purified from a mixture of **81** and **82** by semipreparative normal phase HPLC (Eluent A,

Appendix I).

81: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.97 (s, 3H), 1.06 (s, 3H), 1.89 (d, J = 1.2 Hz, 3H), 2.13 (d, J = 16.8 Hz, 1H), 2.16 (d, J = 1.0 Hz, 3H), 2.33 (d, J = 16.8 Hz, 1H), 2.66 (d, J = 9.4 Hz, 1H), 5.23 (s, 1H), 5.95 (s, 1H), 5.98 (dd, J = 15.5, 9.4 Hz, 1H), 6.25 (d, J = 15.5 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.8, 23.5, 27.3, 27.9, 36.7, 47.3, 55.9, 98.4, 117.2, 126.5, 133.7, 135.2, 155.8, 160.0, 198.5. UV λ_{max} = 260 nm (hexane). MS (ESI^+) calcd for $C_{15}H_{19}NO$ $[M + H]^+$ 230.1545, found 230.1332.

82: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.98 (s, 3H), 1.08 (s, 3H), 1.92 (d, J = 1.3, 3H), 2.02 (d, J = 1.5, 3H), 2.17 (d, J = 17.1, 1H), 2.36 (d, J = 17.1, 1H), 2.73 (d, J = 9.9, 1H), 5.21 (s, 1H), 5.95 (s, 1H), 5.98 (dd, J = 15.3, 9.9, 1H), 6.80 (d, J = 15.3, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.6, 23.5, 27.4, 27.8, 36.5, 47.5, 56.3, 97.0, 116.6, 126.3, 131.3, 136.1, 155.3, 160.3, 198.5. UV λ_{max} = 260 nm (hexane).

Preparation of (7*E*,9*E*)-α-Ionylideneacetonitriles (83) and (7*E*,9*Z*)-α-Ionylideneacetonitriles (84) from (±)-α-Ionone by WHE Coupling (route A, Scheme 18). To a freshly prepared solution of NaOMe, prepared from Na (5.47 mol) and MeOH (70 mL), was added a solution of diisopropyl cyanomethylphosphonate (47 g of 95% pure ≈ 44.65 g, 0.218 mol) in TBME (20 mL) at 0-5°C in 20 min under N_2. After stirring at room temperature for 1 h, the mixture was cooled down in an ice bath and freshly distilled (±)-α-ionone (38 g, 0.20 mol) in TBME (20 mL) was added in 45 min at 0-5°C. The mixture was stirred at room temperature for 4 h and the product was quenched with water, diluted with TBME, washed with brine, and dried over Na_2SO_4. After solvent evaporated under reduced pressure, 45 g of a pale yellow

oil was obtained. The crude product was purified by fractional distillation to yield a mixture of (**7*E*,9*E*)-83** and (**7*E*,9*Z*)-84** (b.p. = 107-110°C at 10 mm) as a colorless oil (31.6 g, 0.147 mol, 74%); **83**:**84** = 3:1 (determined by analytical normal phase HPLC, Eluent A, See Appendix I). Analytically pure samples of **83** and **84** were separated by semipreparative normal phase HPLC (Eluent B, Appendix I).

83: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.81 (s, 3H), 0.91 (s, 3H), 1.20 (m, 1H), 1.46 (m, 1H), 1.55 (d, J = 2.0 Hz, 3H), 2.02 (m, 2H), 2.13 (d, J = 1.0 Hz, 3H), 2.22 (d, J = 9.8 Hz, 1H), 5.15 (s, 1H), 5.46 (m, 1H), 5.94 (dd, J = 15.5, 9.4 Hz, 1H), 6.12 (d, J = 15.5 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.9, 140.5, 135.6, 132.7, 131.5, 122.1, 96.3, 54.6, 32.5, 31.3, 27.7, 26.8, 23.0, 22.8, 16.8. UV λ_{max} = 260 nm (hexane); MS (DEI$^+$) calcd for $C_{15}H_{21}N$ $[M + H]^+$ 216.1752, found 216.1831.

84: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.83 (s, 3H), 0.92 (s, 3H), 1.20 (m, 1H), 1.46 (m, 1H), 1.57 (d, J = 2.0 Hz, 3H), 2.02 (m, 2H), 2.14 (d, J = 1.0 Hz, 3H), 2.30 (d, J = 10 Hz, 1H), 5.08 (s, 1H), 5.46 (m, 1H), 5.96 (dd, J = 15.6, 9.6 Hz, 1H), 6.65 (d, J = 15.6 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.5, 141.3, 136.1, 132.8, 128.9, 121.9, 94.9, 54.8, 32.4, 31.5, 27.8, 26.8, 23.0, 22.8, 16.5. UV λ_{max} = 258 nm (hexane); MS (DEI$^+$) calcd for $C_{15}H_{21}N$ 215.1674, found 215.2000.

Synthesis of (7*E*,9*E*)-α-Ionylideneacetonitriles (83) and (7*E*,9*Z*)-α-Ionylideneacetonitriles (84) from (±)-α-Ionone and Cyanoacetic Acid (route B, Scheme 18). Freshly distilled (±)-α-ionone (32.0 g, 0.166 mol) was transferred into a 250 mL three necked flask using cyclohexylamine (55 mL, 48 g, 0.48 mol). Cyanoacetic acid (18 g, 0.21 mol) was added and the mixture was heated at 80-85°C

under N_2. After 3.5 h, the mixture was allowed to cool down to room temperature and the product was partitioned between hexane (150 mL) and water (150 mL). The organic layer was removed and the aqueous layer was extracted with hexane (50 mL). The combined organic layer was washed with water (3 X 200 mL), dried over Na_2SO_4, and evaporated to dryness to give 34 g of a pale yellow oil. The crude product was purified by fractional distillation to yield a mixture of **83** and **84** (b.p. = 105-110°C at 10 mm) as a colorless oil (26.7 g, 0.124 mol, 75%) that was shown by ^{1}H-NMR as well as analytical normal phase HPLC (Eluent A, Appendix I) to consist of **83** (92%) and **84** (8%) [ratio of isomeric mixture: **83**: (*7E,9E*) / **84**: (*7E,9Z*) = 12/1]. The NMR data were shown to be identical with **83**/**84** characterized earlier.

Palladium(II)-Mediated Oxidation of (±)-α-Ionylideneacetonitrile (83:84 = 12:1) to (*7E,9E*)-3-Keto-α-Ionylideneacetonitrile (81) (Table 2, Entry 1). A solution of (±)-α-ionylideneacetonitrile **83/84** (19.6 g, 91.0 mmol, **83**:**84** = 12:1) in dichloromethane (150 mL) was cooled down in an ice-salt bath to 0°C under N_2 and was treated with K_2CO_3 (8.4 g, 61 mmol) and Pd/C (10 wt.% on C, 7.5 g ~ 0.75 g Pd, 7.1 mmol). A 5.5 M anhydrous solution of TBHP in decane (100 mL, 0.550 mol) was added to the mixture dropwise while maintaining the temperature at 0°C. The mixture was stirred for 36 h at 0°C and 50 h at R.T. under N_2. The solids were removed by filtration through celite and the filtrate was washed with water (3 X 150 mL), brine, and dried over Na_2SO_4. The solvent was removed under reduced pressure to give 24 g of a yellow oil. The crude product was purified by column chromatography (hexane:ethyl acetate, from 98:2 to 92:8) to yield a mixture of **81** and **82** (11 g, 48

mmol, 53%) as a yellow oil. The product was shown by analytical normal phase HPLC (Eluent A, Appendix I) to consist of an isomeric mixture of **81**:**82** = 12:1. Crystallization from ethanol at -20°C gave the (*7E,9E*)-isomer (**81**) as a white crystal (6.0 g, 26 mmol, 29%). The NMR data were shown to be identical with that of **81** characterized previously.

Bleach Oxidation of (±)-α-Ionylideneacetonitrile (83:84 = 12:1) to (*7E,9E*)-3-Keto-α-Ionylideneacetonitrile (81) (Table 2, entry 3). (±)-α-Ionylideneacetonitriles (**83**/**84**) (27 g, 0.12 mol; 83:84 = 12:1) was transferred into a 1 L three-necked flask using acetonitrile (103 mL, 81.0 g, 197 mmol). K_2CO_3 (1.71 g, 12.4 mmol) was added and the mixture was cooled down in an ice-salt bath to 0°C under N_2. A 70% solution of TBHP in water (124 mL, 112 g, 70% ≈ 78.1 g, 867 mmol) was added dropwise to the mixture under N_2 at 0 °C in 30 min. Household bleach containing 5.25% NaOCl (386 g, 20.3 g NaOCl, 272 mmol) was then added over a period of 8 h at -5 to 0°C. After the addition was completed, the reaction mixture was stirred at 0°C for an additional hour. The product was extracted with hexane (200 mL) and the organic layer was separated. The water layer was washed with hexane (2 X 100 mL) and the combined organic layer was washed with water (3 X 200 mL), dried over Na_2SO_4, and evaporated to give 36.7 g of a yellow oil. The crude product was purified by column chromatography (hexane:ethyl acetate, from 98:2 to 92:8) to yield a mixture of (*7E,9E*)-3-keto-α-ionylideneacetonitrile (**81**) and (*7E,9Z*)-3-keto-α-ionylideneacetonitrile (**82**) (15.1 g, 65.6 mmol, 53%) as a yellow oil. The product was shown by analytical normal phase HPLC (Eluent A, Appendix I) and ^{1}H NMR to

consist of **81** (92%) and **82** (8%) [**81**: (*7E,9E*) / **82**: (*7E,9Z*) = 12:1]. Crystallization from ethanol at -15°C gave **81** as a white crystal (10.5 g, 45.79 mmol, 37%). The ^{1}H-NMR spectrum of this white solid was identical with that of **81** characterized earlier.

Reduction of (*7E,9E*)-3-Keto-α-Ionylideneacetonitrile (81) to (*7E,9E*)-3-Hydroxy-α-Ionylideneacetonitriles (77 – 80) with $NaBH_4$ (Table 3, Entry 1). To a solution of (*7E,9E*)-3-keto-α-ionylideneacetonitrile (**81**) (2.0 g, 8.7 mmol) in 20 mL ethanol and 15 mL water was added $NaBH_4$ (0.7 g, 17 mmol) at 0°C. The mixture was allowed to warm up to room temperature, stirred for 24 h, and the product was treated with 10 mL of 0.3 N HCl and extracted with ethyl acetate (50 mL). The organic layer was removed and the aqueous layer was extracted with 30 mL of ethyl acetate. The combined organic layer was washed with 5% $NaHCO_3$, water, and dried over Na_2SO_4. After solvent evaporation, the crude product was purified by column chromatography (hexane:acetone = 97:3) to afford 3-hydroxy-α-ionylidene-acetonitriles **77** – **80** (1.95 g, 8.43 mmol, 97%) as a colorless oil. A mixture of **77**+**78** was separated from **79**+**80** by semipreparative HPLC and was fully characterized by ^{1}H and ^{13}C NMR as well as mass spectrometry and UV-visible spectrophotometry. The isomeric ratio of (**77**+**78**):(**79**+**80**) = 1:1 was established by normal phase HPLC (Eluent A, Appendix I) of the mixture. Hydroxynitriles **77**+**78** and **79**+**80** were each shown by chiral HPLC (Eluent C, Appendix I) to consist of an approximately 1:1 mixture of enantiomers.

(*7E,9E*)-(3,6-*trans*)-3-Hydroxy-α-ionylideneacetonitriles (77+78): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.84 (s, 3H), 0.99 (s, 3H), 1.37 (dd, J = 13.3, 6.4, 1H), 1.59 (s, 3H), 1.81 (dd, J = 13.3, 6.4, 1H), 2.13 (s, 1H), 2.44 (d, J = 10.0, 1H), 4.25 (m, 1H), 5.17 (s,

1H), 5.58 (s, 1H), 5.85 (dd, *J* = 15.4, 10.0, 1H), 6.15 (d, *J* = 15.4, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.8, 22.7, 24.3, 29.3, 34.0, 44.1, 54.7, 65.4, 96.9, 117.6, 125.5, 133.0, 135.9, 138.5, 156.4. UV λ_{max} = 260 nm (hexane). MS (FAB^+) calculated for $C_{15}H_{21}NO$ $[M+H]^+$ 232.1701, found 232.2000.

***(7E,9E)*-(3,6-*cis*)-3-Hydroxy-α-ionylideneacetonitriles (79+80):** ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.83 (s, 3H), 0.94 (s, 3H), 1.35 (dd, *J* = 12.9, 9.8, 1H), 1.60 (t, *J* = 1.5, 3H), 1.64 (dd, *J* = 12.9, 6.5, 1H), 2.12 (d, *J* = 1.0, 3H), 2.19 (d, *J* = 9.3, 1H), 4.23 (m, 1H), 5.17 (s, 1H), 5.53 (s, 1H), 5.95 (dd, *J* = 15.3, 9.3, 1H), 6.14 (d, *J* = 15.3, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.8, 22.4, 26.9, 29.1, 34.9, 40.5, 54.6, 66.3, 96.8, 117.6, 125.8, 131.9, 136.0, 139.2, 156.7. UV λ_{max} = 260 nm (hexane). MS (FAB^+) calculated for $C_{15}H_{21}NO$ $[M+H]^+$ 232.1701, found 232.2000.

Reduction of (*7E,9E*)-3-Keto-α-Ionylideneacetonitrile (81) to (*7E,9E*)-3-Hydroxy-α-Ionylideneacetonitriles (77 – 80) with Triisobutylaluminum (TIBA) (Table 3, Entry 2). A solution of (*7E,9E*)-3-keto-α-ionylideneacetonitrile (**81**) (0.15 g, 0.65 mmol) in toluene (10 mL) was cooled down to -40°C under N_2 and a solution of triisobutylaluminum (3 mL of 1M in toluene, 3 mmol) was added. The course of the reaction was monitored by HPLC. The mixture was allowed to warm up to R.T. and stirred for 1 h. The reaction was quenched by adding a dilute aqueous solution of HCl (0.5 mL, 5% v/v) followed by water (10 mL). The product was diluted with TBME (10 mL) and washed sequentially with brine and water. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The product (0.14 g, 0.62 mmol, 95%) was shown by HPLC to consist of two fractions which were separated by semipreparative

HPLC (Eluent A, Appendix I) and identified in the order of chromatographic elution as (*7E,9E*)-3-hydroxy-α-ionylideneacetonitriles **77**+**78** (40%) and (*7E,9E*)-3-hydroxy-α-ionylideneacetonitriles **79**+**80** (60%). The identification was accomplished by comparison of the ^{1}H-NMR spectra and the HPLC retention times of the hydroxynitriles with those of authentic samples characterized earlier. Hydroxynitriles **77**+**78** and **79**+**80** were each shown by chiral HPLC (Eluent C, Appendix I) to consist of an approximately 1:1 mixture of enantiomers.

Reduction of (*7E,9E*)-3-Keto-α-Ionylideneacetonitrile (81) to (*7E,9E*)-3-Hydroxy-α-Ionylideneacetonitriles (77 – 80) with Sodium Borohydride/*dl*-Tartaric acid (Table 3, Entry 6). A solution of *dl*-tartaric acid (46 mg, 0.31 mmol) in EtOH (4 mL) was cooled down to 0°C and was treated with $NaBH_4$ (12 mg, 0.32 mmol). After the evolution of H_2 subsided, the mixture was stirred at R.T. for 1 h and was then cooled down to -15°C and treated with a solution of (*7E,9E*)-3-keto-α-ionylideneacetonitrile (**81**) (72 mg, 0.31 mmol) in EtOH (3 mL). $NaBH_4$ (24 mg, 0.63 mmol) in EtOH (3 mL) was added to the suspension at -15°C and the course of the reaction was followed by HPLC (Eluent A, Appendix I). After 2h, the product was partitioned between water (10 mL) and ethyl acetate (15 mL). The organic layer was removed and the aqueous layer was extracted with ethyl acetate (10 mL). The combined organic layer was washed with water (2 X 10 mL), dried over Na_2SO_4, and evaporated to dryness. The crude product (68 mg, 0.29 mmol, 94%) was shown by HPLC to consist of two major fractions which were separated by semipreparative HPLC and identified in the order of chromatographic elution as (*7E,9E*)-3-hydroxy-α-

ionylideneacetonitriles **77**+**78** (70%) and (*7E,9E*)-3-hydroxy-α-ionylidene-acetonitriles **79**+**80** (30%). The ^{1}H-NMR and the HPLC retention times of the hydroxynitriles were identical with those of authentic samples of these compounds characterized earlier. Hydroxynitriles **77**+**78** and **79**+**80** were each shown by chiral HPLC (Eluent C, Appendix I) to consist of an approximately 1:1 mixture of enantiomers.

Reduction of **81** to **77 – 80** with sodium borohydride/2,3-dibenzoyl-*d*-tartaric acid, sodium borohydride/*d*-tartaric acid, sodium borohydride/*l*-tartaric acid were carried out employing the same experimental procedure and produced similar results (Table 3, Entry 3-5).

Reduction of (*7E,9E*)-3-Keto-α-Ionylideneacetonitrile (81) to Hydroxynitrile 77 – 80 with Sodium bis(2-methoxyethoxy)aluminum Hydride (Red-Al™) (Table 3, Entry 7). A solution of (*7E,9E*)-3-keto-α-ionylideneacetonitrile (**81**) (0.12 g, 0.52 mmol) in TBME (5 mL) was cooled down to -5°C under N_2, a solution of Red-Al™ (0.18 mL of 0.65 wt.% in toluene, 0.12 g, 0.59 mmol) in TBME (1 mL) was added, and the mixture stirred for 1 h at this temperature. The reaction was quenched by adding water (10 mL) and the product was extracted with TBME (10 mL) and washed sequentially with brine and water. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The product (0.12 g, 0.50 mmol, 95%) was shown by HPLC to consist of (*7E,9E*)-3-hydroxy-α-ionylideneacetonitriles **77**+**78** (57%) and (*7E,9E*)-3-hydroxy-α-ionylideneacetonitriles **79**+**80** (43%). The identification was accomplished by comparison of the HPLC retention times and UV spectra of the hydroxynitriles

obtained by a photodiode array detector with those of authentic samples characterized earlier. Hydroxynitriles **77**+**78** and **79**+**80** were each shown by chiral HPLC (Eluent C, Appendix I) to consist of an approximately 1:1 mixture of enantiomers.

General Procedure for Reduction of 81 to 77 – 80 with Selectrides (Table 3, Entry 8 – 11).

Reduction of (*7E,9E*)-3-Keto-α-Ionylideneacetonitrile (81) to Hydroxynitriles 77 – 80 with Potassium tri-*sec*-Butylborohydride (K-Selectride™) (Table 3, Entry 11). A solution of (*7E,9E*)-3-keto-α-ionylideneacetonitrile (**81**) (3.0 g, 13 mmol) in TBME (25 mL) was cooled down to -30°C under N_2, a solution of K-Selectride™ (20 mL of 1 M in THF, 20 mmol) in TBME (10 mL) was added dropwise in 40 min and the mixture was stirred at this temperature for 4 h. The reaction mixture was treated with 15 mL of 3 N NaOH followed by 15 mL of 30% H_2O_2 and stirred at R.T. for 30 min. The product was extracted with TBME (10 mL) and washed sequentially with brine and water, dried over Na_2SO_4, and evaporated to dryness to give a colorless oil. The product (2.9 g, 12 mmol, 96%) after column chromatography (hexane:acetone = 97:3) was shown by HPLC to consist of a mixture of (*7E,9E*)-3-hydroxy-α-ionylideneacetonitriles **77**+**78** (86%) and (*7E,9E*)-3-hydroxy-α-ionylideneacetonitriles **79**+**80** (14%). These were identified by comparison of their HPLC retention times and UV spectra obtained by a photodiode array detector with those of authentic samples of these hydroxynitriles characterized earlier. Hydroxynitriles **77**+**78** and **79**+**80** were each shown by chiral HPLC (Eluent C) to consist of an approximately 1:1 mixture of enantiomers.

Reduction of 81 with Lithium tri-*sec*-Butylborohydride (L-Selectride™) (Table 3, Entry 8). Ketonitrile **81** was reduced using the same general experimental procedure as described above. The product (94 mg, 0.41 mmol, 93%) was worked up as described earlier and was shown by HPLC to consist of (*7E,9E*)-3-hydroxy-α-ionylideneacetonitriles **77+78** (55%) and (*7E,9E*)-3-hydroxy-α-ionylideneacetonitriles **79+80** (45%). Hydroxynitriles **77+78** and **79+80** were each shown by chiral HPLC to consist of an approximately 1:1 mixture of enantiomers.

Reduction of 81 with Sodium tri-*sec*-Butylborohydride (N-Selectride™) (Table 3, Entry 9). The reaction was carried out and worked up as described in the general procedure to give a mixture of (*7E,9E*)-3-hydroxy-α-ionylideneacetonitriles **77**+**78** (71%) and (*7E,9E*)-3-hydroxy-α-ionylidene-acetonitriles **79**+**80** (29%) [(94 mg, 0.41 mmol, 92%)]. Hydroxynitriles **77**+**78** and **79**+**80** were each shown by chiral HPLC to consist of an approximately 1:1 mixture of enantiomers.

Reduction of 81 with Potassium Trisiamylborohydride (KS-Selectride™) (Table 3, Entry 10). The reaction was carried out and worked up as described in the general procedure to give a mixture of (*7E,9E*)-3-hydroxy-α-ionylideneacetonitriles **77**+**78** (69%) and (*7E,9E*)-3-hydroxy-α-ionylidene-acetonitriles **79**+**80** (31%) [(90 mg, 0.39 mmol, 91%)]. Hydroxynitriles **77**+**78** and **79**+**80** were each shown by chiral HPLC to consist of an approximately 1:1 mixture of enantiomers.

Reduction of (*7E,9E*)-3-Keto-α-Ionylideneacetonitrile (81) to Hydroxynitriles (77 – 80) with (*R*)-2-Methyl-CBS-Oxazaborolidine (Table 3, Entry 12). To a solution of (*R*)-2-methyl-CBS-oxazaborolidine (0.3 mL 1M in toluene, 0.3 mmol) in TBME (4 mL) was added BH_3.THF (0.3 mL 1M in THF, 0.3 mmol) at R.T. under N_2. The mixture was stirred at R.T. for 20 min and was then cooled down to 0°C and treated with a solution of (*7E,9E*)-3-keto-α-ionylideneacetonitrile (**81**) (69 mg, 0.30 mmol) in TBME (3 mL). After stirring the reaction mixture for 1.5 h at 0°C, HPLC (Eluent A, Appendix I) showed the complete reduction of **81**. The reaction was quenched by slow addition of methanol (1 mL) and the product was diluted with TBME, washed with a saturated solution of NH_4Cl, followed by 5% $NaHCO_3$, and then brine. The organic layer was washed with water (10 mL), dried over Na_2SO_4, and evaporated to dryness. After purification by column chromatography purification (hexane:acetone = 97:3), the product (62 mg, 0.27 mmol, 90%) was shown by HPLC to consist of a mixture of (*7E,9E*)-3-hydroxy-α-ionylideneacetonitriles **77**+**78** (14%) and (*7E,9E*)-3-hydroxy-α-ionylideneacetonitriles **79**+**80** (86%). The hydroxynitriles **77**+**78** and **79**+**80** were each shown by chiral HPLC to consist of an approximately 1:1 mixture of enantiomers.

Reduction 81 to 77 – 80 with (*S*)-2-Methyl-CBS-Oxazaborolidine (Table 3, Entry 13). The reduction was carried out using the same procedure described above. After work up, a colorless oil (65 mg, 0.28 mmol, 93%) was obtained that was shown by HPLC to consist of a mixture of (*7E,9E*)-3-hydroxy-α-ionylideneacetonitriles **77+78** (25%) and (*7E,9E*)-3-hydroxy-α-ionylideneacetonitriles **79+80** (75%). The

hydroxynitriles **77**+**78** and **79**+**80** were each shown by chiral HPLC to consist of an approximately 1:1 mixture of enantiomers.

Reduction of (*7E,9E*)-3-Hydroxy-α-Ionylideneacetonitrile (77 – 80) to (*7E,9E*)-3-Hydroxy-α-Ionylideneacetaldehydes (73 – 76) with DIBAL-H. A solution of hydroxynitriles **77+78** (86%) and **79+80** (14%) [2.3 g, 10 mmol] in CH_2Cl_2 (10 mL) was cooled down to -40°C under N_2 and a 1M solution of DIBAL-H in CH_2Cl_2 (33 mL, 33 mmol) was added dropwise in one hour. After the addition was completed, the reaction mixture was allowed to stir at -30°C for 1 h. The mixture was then treated with a very slow addition of a homogeneous mixture of 26 g of water absorbed on n-silica (0.3 g of water/g of silica) at a rate that the temperature remained below -10°C [caution: the addition of silica/water results in rapid elevation of the temperature]. After the addition was completed, the reaction mixture was allowed to stir at 0°C for 2 h. Na_2SO_4 (3 g) was added and the solids were filtered off and washed with CH_2Cl_2 (20 mL). The organic layer was washed with water, dried over Na_2SO_4, and evaporated to dryness to give a pale yellow oil (2.7 g). Column chromatography (hexane:ethyl acetate, 95:5 to 80:20) of the product gave two fractions as **73**+**74** (1.2 g, 4.9 mmol, 49%) and **75**+**76** (0.49 g, 2.1 mmol, 21%).

***trans*-3-Hydroxy-α-ionylideneacetaldehydes (73+74):** ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (s, 3H), 1.03 (s, 3H), 1.40 (dd, J = 13.3, 6.8, 1H), 1.62 (s, 3H), 1.85 (dd, J = 13.3, 5.8, 1H), 2.00 (d, J= 1.3, 1H), 2.26 (d, J = 1.0, 3H), 2.50 (d, J = 10, 1H), 4.27 (s, 1H), 5.61 (s, 1H), 5.93 (d, J = 8.0, 1H), 6.02 (dd, J = 15.6, 10.0, 1H), 6.23 (d, J = 15.6, 1H), 10.12 (d, J = 8.0, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.3, 22.7, 24.4,

29.4, 34.1, 44.3, 55.0, 65.4, 125.3, 128.9, 133.6, 136.0, 136.3, 138.3, 153.9, 191.4. UV λ_{max} = 280 nm (hexane). HRMS (EI^+) calculated for $C_{15}H_{22}O_2$ 234.1620, found 234.1706.

***cis*-3-Hydroxy-α-ionylideneacetaldehydes (75+76):** ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86 (s, 3H), 0.96 (s, 3H), 1.39 (dd, *J* = 12.3, 9.9, 1H), 1.63 (d, *J* = 0.8, 3H), 1.68 (dd, *J* = 12.3, 6.4, 1H), 2.25 (d, *J* = 9.3, 1H), 2.25 (s, 3H), 4.26 (m, 1H), 5.55 (s, 1H), 5.92 (d, *J* = 8.3, 1H), 6.11 (dd, *J* = 15.6, 9.3, 1H), 6.22 (d, *J* = 15.6, 1H), 10.11 (d, *J* = 8.3, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.3, 22.5, 27.0, 29.2, 34.9, 40.8, 55.1, 66.4, 125.6, 128.9, 135.0, 136.4, 139.2, 154.3, 191.5. UV λ_{max} = 282 nm (hexane). MS (ESI^+) calculated for $C_{15}H_{22}O_2$ $[M+H-H_2O]^+$ 217.1592, found 217.2000.

One-Pot Reduction of (*7E,9E*)-3-Keto-α-Ionylideneacetonitrile (81) to Hydroxyaldehydes 73 – 76 with Potassium tri-*sec*-butylborohydride (K-Selectride™) Followed by DIBAL-H. A solution of (*7E,9E*)-3-keto-*α*-ionylideneacetonitrile (**81**) (1.2 g, 5.2 mmol) in TBME (10 mL) was cooled down to -30°C under N_2, A solution of K-Selectride™ (7.6 mL of 1 M in THF, 7.6 mmol) in TBME (5 mL) was added dropwise in 30 min and the mixture was stirred at this temperature and the course of the reaction was monitored by HPLC (Eluent A, Appendix I). After 2 h, **81** was shown by HPLC to have converted to a mixture of (*7E,9E*)-3-hydroxy-*α*-ionylideneacetonitriles **77**+**78** (86%) and (*7E,9E*)-3-hydroxy-α-ionylideneacetonitriles **79**+**80** (14%). The reaction mixture was then treated with a 1M solution of DIBAL-H in CH_2Cl_2 (13 mL, 13 mmol) dropwise in 30 minutes. After the addition was completed, the reaction mixture was allowed to stir at -20°C for 3 h.

The product was then treated with a very slow addition of a homogeneous mixture of 20 g of water absorbed on n-silica (0.5 g of water/g of silica) at a rate that the temperature remained below -10°C [caution: the addition of silica/water results in rapid elevation of the temperature]. The reaction mixture was allowed to stir at 0°C for 2 h. Na_2SO_4 (3 g) was added and the solids were filtered off and washed with CH_2Cl_2 (20 mL). The organic layer was washed with water, dried over Na_2SO_4, and evaporated to dryness to give a pale yellow oil (1.9 g). Column chromatography (hexane:ethyl acetate, 95:5 to 80:20) of the product gave two fractions as **73**+**74** (0.94 g, 4.0 mmol, 77%) and **75**+**76** (0.077 g, 0.33 mmol, 6%) that were shown by ^{1}H-NMR to be identical with previous characterized samples.

Oxidative Degradation of (*3R,3'R,6'R*)-Lutein Diacetate to (*3R,6R*)-3-Hydroxy-13-Apo-ε-Caroten-13-One (119) and (*3R*)-3-Hydroxy-β-Ionone (42).

Preparation of (3R,3'R,6'R)-Lutein Diacetate. Naturally occurring (*3R,3'R,6'R*)-lutein (**1**) was obtained from Kemin Health (Des Moines, Iowa) and converted to (*3R,3'R,6'R*)-lutein diacetate as follows. A solution of (*3R,3'R,6'R*)-lutein (**1**) (3.0 g, 75% pure ≈ 2.3 g, 4.0 mmol) in 20 mL of THF was treated with pyridine (2.5 mL, 2.5 g, 30 mmol) and acetic anhydride (2.5 mL, 2.7 g, 27 mmol) and the mixtue was heated at 45°C under N_2 overnight. The product was partitioned between water (50 mL) and hexane (50 mL). The organic layer was removed and washed sequentially with 50 mL of aqueous HCl (5%, v/v), 50 mL of saturated sodium bicarbonate solution, and water (50 mL). The organic layer was dried over Na_2SO_4 and evaporated to dryness to give a red solid which was purified by column chromatography on n-

silica (hexane:acetone, from 90:10 to 70:30) to give lutein diacetate (2.3 g, 3.5 mmol; 89%).

Oxidative Degradation of (3R,3'R,6'R)-Lutein Diacetate. A solution of (*3R,3'R,6'R*)-lutein diacetate (1.0 g, 1.5 mmol) in ethyl acetate (30 mL) was cooled down in an ice-salt bath to 0°C under N_2 and was treated with a 70% solution of TBHP in water (2.7 mL, 2.4 g 70% ≈ 1.7 g, 19 mmol). Household bleach containing 5.25% NaOCl (8.8 g, 0.46 g NaOCl, 6.2 mmol) was then added over a period of 20 min at 0°C. After the addition was completed, the reaction mixture was allowed to warm up to R.T. and stirred for 3 h. The organic layer was removed and the water layer was washed with EtOAc (2 X 100 mL). The combined organic layer was washed with water (2 X 150 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was dissolved in dichloromethane (30 mL) and saponified with KOH/MeOH (30 mL, 10%, wt/v) at R.T. under N_2. After 2 h, the product was washed with water (3 X 100 mL), dried over Na_2SO_4, and evaporated to dryness. Purification by column chromatography on n-silica (hexane:acetone, from 95:5 to 70:30) followed by semipreparative HPLC (Eluent A) afforded two major products which were fully characterized from their UV-Vis, CD, ^{1}H- and ^{13}C-NMR, and mass spectra as (*3R,6R*)-3-hydroxy-13-apo-ε-caroten-13-one (**119**) and (*3R*)-3-hydroxy-β-ionone (***3R*-42**).

(*3R,6R*)-3-Hydroxy-13-apo-ε-caroten-13-one (119): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.84 (s, 3H), 0.99 (s, 3H), 1.37 (dd, *J* = 13.3, 6.8, 1H), 1.60 (s, 3H), 1.82 (dd, *J* = 13.3, 6.0, 1H), 1.99 (s, 3H), 2.28 (s, 3H), 2.43 (d, *J* = 9.5, 1H), 4.24 (s, 1H), 5.56 (s, 1H), 5.68 (dd, *J* = 15.6, 10.0, 1H), 6.16 (d, *J* = 15.3, 1H), 6.16 (m, 2H), 7.52 (dd, *J* =

11.8, 15.3, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.4, 22.7, 24.2, 27.6, 29.4, 34.0, 44.4, 54.9, 65.6, 125.0, 127.7, 129.7, 133.6, 136.8, 136.9, 139.0, 144.4, 198.5. UV λ_{max} = 322 nm (hexane). CD: 320 nm (10.6 mdeg). MS (ESI^+) calculated for $C_{18}H_{26}O_2$ $[M+Na]^+$ 297.1830, found 297.1720.

(*3R*)-3-Hydroxy-β-ionone (*3R*-42): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.11 (s, 3H), 1.12 (s, 3H), 1.25 (s, 1H), 1.49 (t, *J* = 12.0, 1H), 1.77 (s, 3H), 1.80 (dd, *J* = 2.0, 3.6, 1H), 2.09 (dd, *J* = 9.5, 17.5, 1H), 2.30 (s, 3H), 2.43 (dd, *J* = 5.6, 17.5, 1H), 4.00 (m, 1H), 6.11 (d, *J* = 16.5, 1H), 7.21 (d, *J* = 16.5, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.6, 27.3, 28.5, 30.0, 36.9, 42.7, 48.4, 64.5, 132.3, 135.6, 142.3, 198.6. UV λ_{max} = 290 nm (ethanol). MS (FAB^+) calculated for $C_{13}H_{20}O_2$ $[M+H]^+$ 208.1541, found 209.1312. CD: 310 nm (2.98 mdeg), 270 nm (-2.87 mdeg).

Enzyme-Mediated Acylation of (*7E,9E*)-3-Hydroxy-α-Ionylidene-acetaldehydes 73+74 with Lipase AK (*pseudomonas fluorescens*). To a solution of (*7E,9E*)-3-hydroxy-α-ionylideneacetaldehydes **73+74** (2.4 g, 10.32 mmol) in 20 mL of pentane was added 1.5 g of lipase AK (*pseudomonas fluorescens*) and vinyl acetate (2.84 mL, 2.65 g, 30.78 mmol). The mixture was refluxed (35-36°C) under N_2 and the course of the enzymatic acylation was monitored by chiral HPLC (Eluent D, Appendix I). After 48 h, the product was filtered through celite and the filtrate was evaporated to dryness to give a yellow oil (2.7 g). Column chromatography (hexane:ethyl acetate, 98:2 to 85:15) of the product gave two major fractions.

Without characterization, the first fraction was dissolved in CH_2Cl_2 (25 mL) and treated with KOH/MeOH (2.3 mL, 10% wt/v) for 2 h at 0°C. The product was washed

with water (3 X 50 mL), dried over Na_2SO_4, and evaporated to dryness. The product was fully characterized from its UV, CD, ^{1}H- and ^{13}C-NMR, and mass spectra as (*3S,6S*)-3-hydroxy-α-ionylideneacetaldehyde (**74**) (1.0 g, 4.3 mmol; 97%). The optical purity of **74** (93% *ee*) was established by chiral HPLC.

The second fraction was fully characterized from its UV, CD, ^{1}H- and ^{13}C-NMR, and mass spectra as (*3R,6R*)-3-hydroxy-α-ionylideneacetaldehyde (**73**) (1.0 g, 4.4 mmol, 43%). The optical purity of **73** (94% *ee*) was established by chiral HPLC.

The absolute configuration of hydroxyaldehydes **73** and **74** was assigned by comparison of their ^{1}H NMR and CD spectra with those of the standard C_{18}-ketone **119** prepared by oxidative degradation of (*3R,3'R,6'R*)-lutein (**1**).

(*3R,6R*)-3-Hydroxy-α-ionylideneacetaldehyde (73): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (s, 3H), 1.03 (s, 3H), 1.40 (dd, *J* = 13.3, 6.8, 1H), 1.62 (s, 3H), 1.85 (dd, *J* = 13.3, 5.8, 1H), 2.0 (d, *J* = 1.3, 1H), 2.26 (d, *J* = 1, 3H), 2.50 (d, *J* = 10, 1H), 4.27 (s, 1H), 5.61 (s, 1H), 5.93 (d, *J* = 8.0, 1H), 6.01 (dd, *J* = 15.6, 10, 1H), 6.23(d, *J* = 15.6, 1H), 10.12 (d, *J* = 8.0, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.3, 22.7, 24.4, 29.4, 34.1, 44.3, 55.0, 65.4, 125.3, 128.9, 136.0, 136.3, 138.3, 153.9, 191.4. UV λ_{max} = 280 nm (hexane). HRMS (EI^+) calculated for $C_{15}H_{22}O_2$ 234.1620, found 234.1706. CD: 281 nm (18 mdeg), 242 nm (-1.3 mdeg).

(*3S,6S*)-3-Hydroxy-α-ionylideneacetaldehyde (74): ^{1}H-NMR and ^{13}C-NMR, UV, and Mass spectra of **74** were identical with those of **73**. CD: 281 nm (-13 mdeg), 242 nm (1.0 mdeg).

Enzyme-Mediated Acylation of (*7E,9E*)-3-Hydroxy-α-Ionylideneacetaldehydes 75+76 with Lipase AK (*pseudomonas fluorescens*). To a solution of (*7E,9E*)-3-hydroxy-α-ionylideneacetaldehydes **75+76** (0.84 g, 3.6 mmol) in 20 mL of pentane was added 0.58 g of lipase AK (*pseudomonas fluorescens*) and vinyl acetate (1.4 mL, 1.3 g, 15 mmol). The mixture was refluxed (35-36°C) under N_2 and the course of the enzymatic acylation was monitored by chiral HPLC (Eluent D, Appendix I). After 50 h, the product was filtered through celite and the filtrate was evaporated to dryness to give a yellow oil (1.0 g). Column chromatography (hexane:ethyl acetate, 98:2 to 85:15) of the product gave two major fractions.

Without characterization, the first fraction was dissolved in CH_2Cl_2 (25 mL) and hydrolyzed with KOH/MeOH (0.80 mL, 10% wt/v) for 2 h at 0°C. After work up, the product was fully characterized from its UV, CD, ^{1}H- and ^{13}C-NMR, and mass spectra as (*3S,6R*)-3-hydroxy-α-ionylideneacetaldehyde (**75**) (0.27 g, 1.1 mmol; 99%). The optical purity of **75** (91% *ee*) was established by chiral HPLC.

The second fraction was similarly characterized from its UV, CD, ^{1}H- and ^{13}C-NMR, and mass spectra as (*3R,6S*)-3-hydroxy-α-ionylideneacetaldehyde (**76**) (0.31 g, 1.3 mmol, 37%). The optical purity of **76** (92% *ee*) was established by chiral HPLC.

The absolute configuration of hydroxyaldehydes **75** and **76** was assigned from comparison of their ^{1}H NMR and CD spectra with those of C_{18}-ketone **119**.

(*3S,6R*)-3-Hydroxy-α-ionylideneacetaldehyde (75): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86 (s, 3H), 0.96 (s, 3H), 1.39 (dd, *J* = 12.3, 9.8, 1H), 1.63 (s, 3H), 1.68 (dd, *J* = 12.3, 6.3, 1H), 2.25 (d, *J* = 9.3, 1H), 2.25 (s, 3H), 4.25 (m, 1H), 5.55 (s, 1H), 5.92 (d, *J* = 8.3, 1H), 6.11 (dd, *J* = 15.6, 9.3, 1H), 6.22 (d, *J* = 15.6, 1H), 10.11 (d, *J* = 8.3, 1H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 13.2, 22.5, 27.0, 29.2, 34.9, 40.8, 55.1, 66.4, 125.6, 128.9, 135.0, 136.4, 139.2, 154.3, 191.5. UV λ_{max} = 282 nm (hexane). MS (ESI^+) calculated for $C_{15}H_{22}O_2$ $[M+H-H_2O]^+$ 217.1592, found 217.2010. CD: 280 nm (17.8 mdeg).

(*3R,6S*)-3-Hydroxy-α-ionylideneacetaldehyde (76): ^{1}H-NMR and ^{13}C-NMR, UV, and Mass spectra were identical with those of **75**. CD: 280 nm (-7 mdeg).

Epimerization of (*3R,6R*)-3-Hydroxy-α-Ionylideneacetaldehydes (73) to (*3S,6R*)-3-Hydroxy-α-Ionylideneacetaldehydes (75). To a solution of optically pure hydroxyaldehyde **73** (3.2 mg, 0.014 mmol) in 1mL acetone and 0.5 mL H_2O was added 0.12 mL of 0.1N HCl. The mixture was stirred at R.T. for 28 hours when HPLC (Eluent A, Appendix I) showed that an equilibrium had established between **73** (32%) and hydroxyaldehyde **75** (68%). The product was washed with water (2 X 10 mL), dried over Na_2SO_4, and evaporated to dryness. Purification by semipreparative HPLC (Eluent A) afforded two major products which were characterized by ^{1}H-NMR and CD as **73** and **75**.

Epimerization of (*3S,6R*)-3-Hydroxy-α-Ionylideneacetaldehydes (75) to (*3R,6R*)-3-Hydroxy-α-Ionylideneacetaldehydes (75). To a solution of optically pure hydroxyaldehyde **73** (4.7 mg, 0.020 mmol) in 1mL acetone and 0.5 mL H_2O was added 0.24 mL of 0.1 N HCl. The mixture was stirred at R.T. for 28 hours when HPLC (Eluent A, Appendix I) showed that the reaction had reached an equilibrium between hydroxyaldehyde **73** (31%) and hydroxyaldehyde **75** (69%). The product was

washed with water (2 X 10 mL), dried over Na_2SO_4, and evaporated to dryness. Purification by semipreparative HPLC (Eluent A) afforded two major products which were characterized by ^{1}H-NMR and CD as **73** and **75**.

General Procedure for the Synthesis of C_{25}-Hydroxyaldehydes 64 – 67.

Synthesis of (*3R,6R*)-3-Hydroxy-12'-Apo-ε-Caroten-12'-Al (64). A solution of (*3R,6R*)-3-hydroxy-α-ionylideneacetaldehyde (**73**) (0.25 g, 1.1 mmol) in MeOH (3 mL) was treated with a solution of the protected Wittig salt **72** (0.82 g, 1.7 mmol) in methanol (2 mL) at R.T. under N_2. 1 mL of a 0.42 M solution of NaOMe (0.42 mmol) in MeOH (freshly prepared from Na in MeOH) was added and the mixture was stirred at R.T. for 4 h. The product was partitioned between water (50 mL) and CH_2Cl_2 (30 mL), the organic layer was removed, and the water layer was extracted with CH_2Cl_2 (20 mL). The combined organic layer was washed with water (2 X 30 mL), dried over Na_2SO_4, and evaporated to dryness to give a red solid (1.3 g). The red solids were dissolved in acetone (4 mL) and water (1 mL) and stirred with 75 μL of 0.3 N HCl for 1 h at R.T. under N_2. The product was extracted with CH_2Cl_2, and sequentially washed with saturated solution of $NaHCO_3$ and water, dried over Na_2SO_4, and evaporated to dryness to give a red oil. Column chromatography (hexane:ethyl acetate, 95:5 to 80:20) gave a red solid that was identified from its UV-visible, CD, ^{1}H- and ^{13}C-NMR, and mass spectra as (*3R,6R*)-3-hydroxy-12'-apo-ε-caroten-12'-al (**64**) (0.33 g, 0.91 mmol; 85%).

Following the above procedure, (*3S,6S*)-3-hydroxy-12'-apo-ε-caroten-12'-al (**65**, 61%), (*3S,6R*)-3-hydroxy-12'-apo-ε-caroten-12'-al (**66**, 53%), and (*3R,6S*)-3-hydroxy-12'-apo-ε-caroten-12'-al (**67**, 69%) were similarly prepared.

(*3R,6R*)-3-Hydroxy-12'-apo-ε-caroten-12'-al (64): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86 (s, 3H), 1.00 (s, 3H), 1.38 (dd, *J* = 13.3, 6.9, 1H), 1.63 (dd, *J* = 2.3, 1.6, 3H), 1.85 (dd, *J* = 13.3, 5.9, 1H), 1.89 (d, *J* = 0.7, 3H), 1.94 (d, *J* = 0.9, 3H), 2.04 (s, 3H), 2.43 (d, *J* = 9.9, 1H), 4.26 (s, 1H), 5.50 (dd, *J* = 15.4, 9.9, 1H), 5.56 (d, *J* = 1.4, 1H), 6.16 (d, *J* = 15.4, 2H), 6.31 (d, *J* = 11.9, 1H), 6.38 (d, *J* = 15.0, 1H), 6.69 (dd, *J* = 14.4, 11.5, 1H), 6.76 (dd, *J* = 15.0, 11.3, 1H), 6.97 (d, *J* = 11.5, 1H), 7.03 (dd, *J* = 11.9, 14.4, 1H), 9.46 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 9.6, 13.0, 13.2, 22.8, 24.2, 29.5, 34.0, 44.6, 55.0, 65.9, 124.6, 127.4, 130.0, 130.3, 131.0, 136.7, 136.9, 137.0, 137.5, 137.7, 141.6, 148.9, 194.5. UV/Vis λ_{max} = 416 nm (ethanol). MS (ESI^+) calculated for $C_{25}H_{34}O_2$ $[M+H]^+$ 367.2637, found 367.2091. CD: 292 nm (+5.82 mdeg), 236 nm (+5.32 mdeg), 212 nm (+4.54 mdeg).

(*3S,6S*)-3-Hydroxy-12'-apo-ε-caroten-12'-al (65): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86 (s, 3H), 1.01 (s, 3H), 1.38 (dd, *J* = 13.3, 6.9, 1H), 1.63 (s, 3H), 1.85 (dd, *J* = 13.3, 5.8, 1H), 1.89 (s, 3H), 1.94 (s, 3H), 2.04 (s, 3H), 2.43 (d, *J* = 9.9, 1H), 4.26 (s, 1H), 5.51 (dd, *J* = 15.4, 9.9, 1H), 5.56 (s, 1H), 6.16 (d, *J* = 15.4, 2H), 6.31 (d, *J* = 11.9, 1H), 6.38 (d, *J* = 15.0, 1H), 6.70 (dd, *J* = 14.4, 11.6, 1H), 6.77 (dd, *J* = 15.0, 11.3, 1H), 6.97 (d, *J* = 11.7, 1H), 7.04 (dd, *J* = 14.4, 12.0, 1H), 9.46 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 9.6, 13.0, 13.2, 22.8, 24.3, 29.5, 34.0, 44.6, 55.0, 65.9, 124.6, 127.4, 130.0, 130.4, 131.0, 136.7, 137.0, 137.5, 137.7, 141.6, 148.9, 194.5. UV/Vis λ_{max} = 416 nm

(ethanol). MS (ESI^+) calculated for $C_{25}H_{34}O_2$ $[M+H]^+$ 367.2637, found 367.2235 $[M+H^+]$; CD: 292 nm (-4.32 mdeg), 238 nm (-4.86 mdeg), 210 nm (-4.72 mdeg).

(*3S,6R*)-3-Hydroxy-12'-apo-ε-caroten-12'-al (66): ^1H NMR (400 MHz, $CDCl_3$) δ 0.86 (s, 3H), 0.95 (s, 3H), 1.40 (dd, *J* = 12.6, 9.8, 1H), 1.65 (s, 3H), 1.66 (m, 1H), 1.89 (s, 3H), 1.94 (s, 3H), 2.04 (s, 3H), 2.18 (d, *J* = 9.4, 1H), 4.25 (m, 1H), 5.50 (s, 1H), 5.61 (dd, *J* = 15.6, 9.4, 1H), 6.15 (d, *J* = 15.6, 1H), 6.16 (d, *J* = 10.8, 1H), 6.31 (d, *J* = 11.9, 1H), 6.37 (d, *J* = 15.2, 1H), 6.69 (dd, *J* = 14.8, 11.8, 1H), 6.76 (dd, *J* = 15.2, 10.8, 1H), 6.97 (d, *J* = 11.8, 1H), 7.03 (dd, *J* = 14.8, 11., 1H), 9.46 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 9.6, 13.0, 13.2, 14.1, 22.6, 27.0, 29.3, 34.8, 40.9, 55.0, 66.7, 124.6, 127.4, 127.5, 130.4, 130.9, 131.0, 136.5, 136.6, 136.9, 137.1, 137.7, 137.8, 141.6, 148.9, 194.5. UV/Vis λ_{max} = 418 nm (ethanol). MS (ESI^+) calculated for $C_{25}H_{34}O_2$ $[M-H]^-$ 365.2480, found 365.2053. CD: 402 nm (+3.73 mdeg), 235 nm (+8.57 mdeg).

(*3R,6S*)-3-Hydroxy-12'-apo-ε-caroten-12'-al (67): ^1H NMR (400 MHz, $CDCl_3$) δ 0.86 (s, 3H), 0.96 (s, 3H), 1.40 (dd, *J* = 12.8, 9.8, 1H), 1.64 (m, 1H), 1.65 (t, *J* = 1.6, 3H), 1.89 (d, *J* = 0.8, 3H), 1.94 (d, *J* = 0.8, 3H), 2.04 (s, 3H), 2.18 (d, *J* = 9.3, 1H), 4.25 (m, 1H), 5.50 (s, 1H), 5.61 (dd, *J* = 15.4, 9.3, 1H), 6.15 (d, *J* = 15.4, 1H), 6.16 (d, *J* = 11.0, 1H), 6.31 (d, *J* = 11.9, 1H), 6.38 (d, *J* = 15.0, 1H), 6.69 (dd, *J* = 14.4, 11.7, 1H), 6.77 (dd, *J* = 15.0, 11.0, 1H), 6.97 (d, *J* = 11.7, 1H), 7.04 (dd, *J* = 14.4, 11.9, 1H), 9.46 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 9.6, 13.0, 13.2, 14.1, 22.6, 27.0, 29.3, 34.8, 41.0, 55.0, 66.8, 124.6, 127.4, 127.5, 130.4, 130.9, 131.0, 136.5, 136.6, 136.9, 137.1, 137.7, 137.8, 141.6, 148.9, 194.5. UV/Vis λ_{max} = 416 nm (ethanol). MS (ESI^+)

calculated for $C_{25}H_{34}O_2$ $[M-H]^-$ 365.2480, found 365.1954 $[M-H^-]$. CD: 406 nm (-4.32 mdeg), 235 nm (-11.23 mdeg).

General Procedure for the Synthesis of Luteins 1 – 4.

Synthesis of (*3R,3'R,6'R*)-Lutein (1). A solution of (*3R,6R*)-3-hydroxy-12'-apo-ε-caroten-12'-al (**64**) (0.26 g, 0.70 mmol) and (*3R*)-3-hydroxy-(β-ionylideneethyl)-triphenylphosphonium chloride [(***3R***)-**16**] (0.41 g, 0.79 mmol) in CH_2Cl_2 (5 mL) was cooled down to -5°C under N_2. A solution of KOH (0.13 g, 2.3 mmol) in H_2O (0.5 mL) was added and the mixture was stirred for 0.5 h at -5°C and 3 h at R.T. Dichloromethane (20 mL) was added, and the product was washed with water (3 X 10 mL). The organic layer was removed, dried over Na_2SO_4, and evaporated to dryness to give 1 g of a red oil. The crude product was thermally isomerized by refluxing in ethyl acetate for 4h under N_2. After solvent evaporation, the product was purified by column chromatography (hexane:ethylacetate, from 90:10 to 50:50) to give a red solid that was crystallized from hexane:acetone = 4:1 and identified from its UV-visible, CD, ^{1}H- and ^{13}C-NMR, and mass spectra as (*3R,3'R,6'R*)-Lutein (**1**) (0.29 g, 0.52 mmol; 74%).

Following the above procedure, (*3R,3'S,6'S*)-lutein (**2**, 82%), (*3R,3'S,6'R*)-lutein or 3'-epilutein (**3**, 85%), and (*3R,3'R,6'S*)-lutein (**4**, 80%) were similarly prepared.

(*3R,3'R,6'R*)-Lutein (1): m.p. 132-134°C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86 (s, 3H), 1.01 (s, 3H), 1.08 (s, 6H), 1.37 (dd, *J* = 13.1, 6.7, 1H), 1.49 (t, *J* = 12.3, 1H), 1.63 (s, 3H), 1.74 (s, 3H), 1.78 (dd, *J* = 12.3, 2.8, 1H), 1.85 (dd, *J* = 13.1, 5.8, 1H), 1.92 (s, 3H), 1.97 (s, 9H), 2.05 (d, *J* = 16.3, 9.7, 1H), 2.40 (dd, *J* = 16.3, 6.7, 1H),

2.41 (d, *J* = 9.9, 1H), 4.01 (m, 1H), 4.26 (s, 1H), 5.44 (dd, *J* = 15.5, 9.9, 1H), 5.55 (s, 1H), 6.12 (m, 2H), 6.17 (m, 3H), 6.26 (m, 2H), 6.37 (m, 2H), 6.63 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.8, 13.1, 21.6, 22.9, 24.3, 28.7, 29.5, 30.3, 34.0, 37.1, 42.6, 44.6, 48.4, 54.9, 65.1, 65.9, 124.5, 124.8, 124.9, 125.6, 126.2, 128.7, 130.0, 130.1, 130.8, 131.3, 132.6, 135.1, 135.7, 136.5, 137.6, 137.7, 138.0, 138.5. UV/Vis λ_{max} = 444 nm (ethanol). HRMS (ESI^+) calculated for $C_{40}H_{56}O_2$ 568.8714, found 568.5973. CD: 284 nm (-1.26 mdeg), 246 nm (+2.67 mdeg), 212 nm (+3.75 mdeg).

(*3R,3'S,6'S*)-Lutein (2): m.p. 128-130°C,^{1}H NMR (400 MHz, $CDCl_3$) δ0.86 (s, 3H), 1.01 (s, 3H), 1.08 (s, 6H), 1.37 (dd, *J* = 13.2, 6.9, 1H), 1.49 (t, *J* = 11.8, 1H), 1.63 (s, 3H), 1.74 (s, 3H), 1.78 (dd, *J* = 11.8, 2.8, 1H), 1.85 (dd, *J* = 13.2, 5.8, 1H), 1.92 (s, 3H), 1.98 (s, 9H), 2.05 (dd, *J* = 16.4, 9.7, 1H), 2.40 (dd, *J* = 16.4, 6.8, 1H), 2.41 (d, *J* = 9.9, 1H), 4.01 (m, 1H), 4.26 (s, 1H), 5.44 (dd, *J* = 15.5, 9.9, 1H), 5.56 (s, 1H), 6.13 (m, 2H), 6.17 (m, 3H), 6.27 (m, 2H), 6.37 (m, 2H), 6.64 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.7, 12.8, 13.1, 21.6, 22.9, 24.3, 28.7, 29.5, 30.2, 34.0, 37.1, 42.5, 44.6, 48.4, 54.9, 65.1, 65.9, 124.5, 124.8, 124.9, 125.6, 126.2, 128.7, 130.0, 130.1, 130.8, 131.3, 132.6, 135.1, 135.7, 136.4, 136.5, 137.6, 137.7, 138.0, 138.5. UV/Vis λ_{max} = 446 nm (ethanol). HRMS (ESI^+) calculated for $C_{40}H_{56}O_2$ 568.8714, found 568.6036. CD: 272 nm (-6.19 mdeg), 238 nm (+0.41 mdeg), 214 nm (-4.73 mdeg).

(*3R,3'S,6'R*)-Lutein (3): m.p. 125-127°C, ^{1}H NMR (400 MHz, $CDCl_3$) δ0.86 (s, 3H), 0.95 (s, 3H), 1.08 (s, 6H), 1.42 (dd, *J* = 11.8, 3.5, 1H), 1.49 (t, *J* = 11.9, 1H), 1.65 (s, 3H), 1.75 (s, 3H), 1.78 (m, 1H), 1.85 (dd, *J* = 12.7, 7.0, 1H), 1.98 (s, 9H), 2.06 (dd, *J* = 16.0, 10.9, 1H), 2.17 (d, *J* = 9.4, 1H), 2.40 (dd, *J* = 16.0, 5.0, 1H), 4.01 (m, 1H),

4.24, (m, 1H), 5.49 (s, 1H), 5.54 (dd, *J* = 15.4, 9.4, 1H), 6.14 (m, 3H), 6.26 (d, 2H), 6.36 (m, 2H), 6.63 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.8, 13.1, 21.6, 22.6, 27.0, 28.7, 29.3, 30.3, 34.8, 37.1, 41.0, 42.5, 48.4, 55.0, 65.1, 66.8, 124.4, 124.8, 124.9, 125.6, 126.2, 128.5, 129.8, 130.1, 130.8, 131.3, 132.5, 132.6, 135.3, 135.7,136.5, 136.7, 137.5, 137.6, 137.7, 138.0, 138.5. UV/Vis λ_{max} = 446 nm (ethanol). HRMS (ESI$^+$) calculated for $C_{40}H_{56}O_2$ $[M+H]^+$ 568.8714, found 568.4256. CD: 333 nm (+1.79 mdeg), 280 nm (-2.71 mdeg), 242 nm (+4.92 mdeg).

(*3R,3'R,6'S*)-Lutein (4): m.p. 123-125°C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86 (s, 3H), 0.95 (s, 3H), 1.08 (s, 6H), 1.41 (m, 1H), 1.49 (t, *J* = 11.9, 1H) 1.65 (s, 3H), 1.75 (s, 3H), 1.80 (m, 1H), 1.92 (m, 1H), 1.98 (s, 9H), 2.05 (dd, *J* = 16.6, 9.0, 1H), 2.17 (d, *J* = 9.4, 1H), 2.40 (dd, *J* = 16.6, 5.5, 1H), 4.01 (m, 1H), 4.24, (m, 1H), 5.50 (s, 1H), 5.54 (dd, *J* = 15.4, 9.4, 1H), 6.16 (m, 5H), 6.26 (d, 2H), 6.36 (m, 2H), 6.63 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.8, 13.1, 21.6, 22.6, 27.0, 28.7, 29.3, 30.3, 34.8, 37.1, 41.0, 42.5, 48.4, 55.0, 65.1, 66.8, 124.4, 124.8, 125.6, 126.2, 129.8, 130.1, 130.8, 131.3, 132.5, 132.6, 135.3, 135.7,136.5, 136.7, 137.5, 137.6, 137.7, 138.5. UV/Vis λ_{max} = 446 nm (ethanol). HRMS (ESI$^+$) calculated for $C_{40}H_{56}O_2$ $[M+H]^+$ 568.8714, found 568.3536. CD: 328 nm (-2.08 mdeg), 266 nm (-5.03 mdeg), 238 nm (-5.78 mdeg).

Preparation of (±)-α-Ionone Ketal (125). Freshly distilled (±)-α-ionone (23 g, 0.11 mol) was transferred into a 250 mL three necked flask with 10 mL hexane and was treated with ethylene glycol (18 mL, 0.32 mol) and trimethylorthoformate (14 mL, 0.13 mol). *p*-Toluenesulfonic acid (0.29 g, 1.5 mmol) was added and the mixture

was stirred at R.T. under nitrogen overnight. The progress of the reaction was monitored by NMR. The product was partitioned between water and hexane, and the organic layer was washed water (3 X 300 mL), dried over Na_2SO_4, and evaporated to dryness to yield a 29.7 g pale yellow oil. The product was identified by NMR as (±)-α-Ionone Ketal (**125**) and was used in the next step without purification. ^{1}H NMR (400MHz, $CDCl_3$) δ 0.81 (s, 3H), 0.88 (s, 3H), 1.17 (m, 1H), 1.41 (dd, J = 13.2, 7.9, 1H), 1.47 (s, 3H), 1.57 (m, 3H), 1.99 (m, 2H), 2.11 (d, J = 9.7, 1H), 3.86 (m, 2H), 3.96 (m, 2H), 5.38 (d, J = 15.4, 1H), 5.40 (s, 1H), 5.61 (dd, J = 15.4, 9.7, 1H). These NMR data were in agreement with the published data.[96]

Oxidation of (±)-α-Ionone Ketal (125) to (±)-3-Keto-α-Ionone Ketal (124). (±)-α-Ionone ketal (**125**) (29.7 g, 12.6 mmol) from preceding experiment was transferred into a 1 L three-necked flask using acetonitrile (105 mL, 82.5 g, 2.00 mol). K_2CO_3 (1.8 g, 13 mmol) was added and the mixture was cooled down in an ice-salt bath to 0°C under N_2. A 70% solution of TBHP in water (108 mL, 97.2 g 70% ≈ 68.0 g, 0.755 mol) was added dropwise to the mixture under N_2 at 0°C in 30 min. Household bleach containing 5.25% NaOCl (356 g, 18.7 g NaOCl, 0.251 mol) was then added over a period of 8 h at -5 to 0°C. After the addition was completed, the reaction mixture was stirred at 0°C for an additional hour. The product was treated with 2g $NaHCO_3$ at 0°C and then extracted with hexane (2 X 150 mL). The combined organic layer was washed with water (3 X 150 mL), dried over Na_2SO_4, and evaporated to dryness to give 30.3 g of pale yellow oil. The crude product was purified by column chromatography (hexane: ethyl acetate, from 98:2 to 85:15) to yield (±)-3-keto-α-

ionone ketal (**124**) (19.0 g, 91.0 mmol, 83%) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.94 (s, 3H), 1.01 (s, 3H), 1.45 (s, 3H), 1.87 (m, 3H), 2.07 (d, J = 16.6, 1H), 2.32 (d, J = 16.6, 1H), 2.53 (d, J = 9.3, 1H), 3.83 (m, 2H), 3.96 (m, 2H), 5.55 (d, J = 15.4, 1H), 5.70 (dd, J = 15.4, 9.3, 1H), 5.89 (d, J = 0.7, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 23.3, 25.0, 27.0, 27.8, 36.0, 47.5, 54.9, 64.4, 106.9, 125.9, 127.6, 134.9, 161.3, 198.9. MS (FAB^+) calculated for $C_{15}H_{22}O_3$ $[M+H]^+$ 251.1647, found 251.1650.

Reduction of (±)-3-Keto-α-Ionone Ketal (124) with $NaBH_4$ (Table 4, Entry 1). To a solution of (±)-3-keto-α-ionone ketal (**124**) (16 g, 66 mmol) in 100 mL ethanol was added $NaBH_4$ (3.8 g, 99 mmol) at 10°C. The mixture was kept at 10°C and allowed to warm up to room temperature, stirred for 4 h, and the product was partitioned between water (400 mL) and ethyl acetate (150 mL). The organic layer was removed and the aqueous layer was extracted with 100 mL of ethyl acetate. The combined organic layer was washed with brine and water, dried over Na_2SO_4, and evaporated to dryness. The crude product (10 g) was deprotected in the following step without purification.

General Procedure for Deprotection of (±)-3-hydroxy-α-ionone ketal. The deprotection of (±)-3-hydroxy-α-ionone ketal to (±)-3-hydroxy-α-ionone (**120 – 123**) was carried out according to the following general procedure in all subsequent reduction reactions.

The crude product (10 g) was transferred into a 500 mL round bottom flask with 100 mL acetone and 20 mL water and the mixture was kept under nitrogen. The solution was treated with 16.5 mL of 0.3N HCl with dropwise addition in 10 minutes

and stirred at R.T. for 3 h. The course of the reaction was monitored by NMR. The crude (±)-3-hydroxy-α-ionone (**120 – 123**) was partitioned between 300 mL water and 150 mL ethyl acetate. The organic layer was washed with saturated $NaHCO_3$, dried over Na_2SO_4, and evaporated to dryness to obtain 13.1 g of a yellow oil. After work up, two fractions were obtained that were separated by column chromatography (hexane: acetone, from 95:5 to 85:15) and identified as *trans*-3-hydroxy-α-ionone (**120+121**) (4.0 g, 19 mmol, 30%) and *cis*-3-hydroxy-α-ionone (**122+123**) (3.9 g, 19 mmol, 28%) in diastereomeric ratio of 1:1 (1.6 g, 7.7 mmol; 77%).

120+121: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.89 (s, 3H), 1.03 (s, 3H), 1.41 (dd, *J* = 13.5, 6.4, 1H), 1.53 (s, 1H), 1.62 (m, 3H), 1.84 (dd, *J* = 13.5, 5.9, 1H), 2.26 (s, 3H), 2.50 (d, *J* = 10.2, 1H), 4.27 (m, 1H), 5.63 (m, 1H), 6.10 (d, *J* = 15.6, 1H), 6.54 (dd, *J* = 15.6, 10.2, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.6, 24.6, 27.2, 29.3, 33.8, 43.8, 54.3, 65.4, 125.8, 133.6, 135.4, 135.2, 147.1, 198.1. UV λ_{max} = 226 nm (hexane). HRMS (EI) m/z calculated for $C_{13}H_{18}O_2$ 206.1307, found 206.1331.

122+123: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (s, 3H), 0.96 (s, 3H), 1.39 (dd, *J* = 12.9, 9.8, 1H), 1.61 (t, *J* = 1.5, 3H), 1.68 (dd, *J* = 12.9, 6.5, 1H), 1.92 (s, 1H), 2.25 (s, 3H), 2.26 (d, *J* = 9.6, 1H), 4.24 (m, 1H), 5.58 (s, 1H), 6.06 (d, *J* = 15.9, 1H), 6.63 (dd, *J* = 15.9, 9.6, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.3, 26.9, 29.0, 34.9, 40.6, 54.2, 66.3, 126.4, 132.6, 134.0, 135.2, 147.8, 198.5. UV λ_{max} = 226 nm (hexane). MS was identical to that of **120+121**.

Reduction of (±)-3-Keto-α-Ionone Ketal (124) with $NaBH_4$/*dl*-Tartaric Acid. A solution of *dl*-tartaric acid (1.8 g, 12 mmol) in ethanol (10 mL) was cooled down to

0°C under N_2 and solid $NaBH_4$ (0.46 g, 12 mol) was added slowly in small portions. An extothermic reaction began with evolution of H_2. The mixture was stirred at R.T. for 20 minutes and was then cooled down to -15°C. (±)-3-Keto-α-ionone ketal (**124**) (2.5 g, 10 mmol) in 8 mL ethanol was added and the mixture was stirred at -15°C for 10 minutes. This was followed by the addition of solid $NaBH_4$ (0.23 g, 6.1 mmol) to the suspension at -15°C. The mixture was allowed to warm up to R.T. and the course of the reaction was followed by HPLC (Eluent A). The product was worked up by pouring the reaction mixture into crushed ice and extraction with ethyl acetate (50 mL). The organic layer was washed with water (2 X 100 mL), dried over Na_2SO_4, and evaporated to dryness to give 2.4 g of a colorless oil identified as ketal **120** – **123**. The oil was dissolved in acetone (25 mL) and water (14 mL) and treated with 1.8 mL of 0.3N HCl to deprotect the ketal as described in the general deprotection procedure. After work up and purification by chromatography, 1.6 g of a colorless oil was obtained that was identified as *trans*-3-hydroxy-α-ionone (**120+121**) and *cis*-3-hydroxy-α-ionone (**122+123**) in diastereomeric ratio of 1:1 (1.6 g, 7.7 mmol; 77%).

Reduction of (±)-3-Keto-α-Ionone Ketal (124) with $NaBH_4$/Cerium Chloride. A solution of (±)-3-keto-α-ionone ketal (**124**) (0.32 g, 1.3 mmol) in methanol (20 mL) was cooled down to -15°C under N_2 and was treated with solid $CeCl_3$ (0.73 g, 2.0 mmol). $NaBH_4$ (80 mg, 2.1 mmol) was added at -15°C and stirred at this temperature for 2 h. The course of the reaction was followed by HPLC (eluent A). The product was poured into a solution of NH_4Cl (10%) and extracted with ethyl acetate (25 mL). The organic layer was washed with water (2 X 80 mL), dried over Na_2SO_4, and

evaporated to dryness to give 0.2 g of ketal **120** – **123** as a colorless oil. The oil was dissolved in acetone (2 mL) and water (4 mL) and treated with 0.15 mL of 0.3N HCl to deprotect the ketal as described in the general procedure. After work up, the crude product was purified by chromatography to afford **120** – **123** (0.10 g, 0.51 mmol; 40%) which was shown by HPLC (eluent A) and NMR to consist of a mixture of *trans*-3-hydroxy-α-ionone (**120+ 121**) (34%) and *cis*-3-hydroxy-α-ionone (**122+ 123**) (66%).

Reduction of (±)-3-Keto-α-Ionone Ketal (124) with 9-BBN. A solution of (±)-3-keto-α-ionone ketal (**124**) (0.2 g, 0.8 mmol) in TBME (6 mL) was cooled down to -35°C under N_2 and a 2M solution of 9-BBN in THF (4 mL, 2 mmol) was added. The mixture was stirred at -40°C for 45 min. The reaction was quenched by addition of water (1 mL) and the mixture was allowed to warm up to R.T. 3N NaOH (1.5 mL) followed by 30% H_2O_2 (1.5 mL) were added and the mixture was stirred for 15 min at R.T. The product was washed with water, dried over Na_2SO_4 and evaporated to dryness to yield ketal **120** – **123** as a colorless oil. The oil was dissolved in acetone (5 mL) and water (1.5 mL) and treated with 0.2 mL of 0.3N HCl to deprotect the ketal as described in the general procedure. After work up with ethyl acetate, the crude product was shown by HPLC (eluent A) to consist of a mixture of 3-keto-α-ionone (**124**) (80%), (3,6)-*trans*-3-hydroxy-α-ionone (8.4%), and (3,6)-*cis*-3-hydroxy-α-ionone (11.6%).

Reduction of (±)-3-Keto-α-Ionone Ketal (124) with Sodium Bis(2-Methoxyethoxy)aluminum Hydride (Red-Al™). A solution of (±)-3-keto-α-ionone

ketal (**124**) (13.3 g, 53.0 mmol) in TBME (30 mL) was cooled down to -20°C under N_2 and a solution of Red-Al™ in toluene (25.0 mL of 65 wt%, 16.8 g, 83.0 mmol) was added dropwise in 40 minutes. The mixture was allowed to warm up to 0°C and stirred for 1 h at this temperature. The reaction was quenched by addition of water (10 mL) at -10°C and stirring for 10 minutes. The product was filtered through celite using acetone. The filtrate was concentrated under reduced pressure and the residue was partitioned between TBME (120 mL) and water (300 mL). The organic layer was removed and sequentially washed with brine and water. After drying over Na_2SO_4 and solvent evaporation, the crude product was dissolved in acetone (50 mL) and water (20 mL) and stirred with 5 mL of 0.3 N HCl at R.T. for 2 h to deprotect the ketal **120** – **123**. After work-up 10.8 g of a yellow oil was obtained. The oil was purified by column chromatography (hexane: ethyl acetate from 90:10 to 70:30) to yield (±)-3-hydroxy-α-ionone (**124**) (6.27 g, 30 mmol, 57%) as a pale yellow oil which was shown by HPLC (Eluent A) and ^{1}H NMR to consist of a 1:1 mixture of (3,6)-*trans*- and (3,6)-*cis*-3-hydroxy-α-ionone.

Reduction of (±)-3-Keto-α-Ionone Ketal (124) with Diisobutylaluminum Hydride (DIBAL-H). A solution of (±)-3-keto-α-ionone ketal (**124**) (0.98 g, 3.9 mmol) in CH_2Cl_2 (7 mL) was cooled down to -30°C under N_2 and a solution of DIBAL-H (7 mL of 1M in CH_2Cl_2, 7 mmol) was added with a syringe in 5 min. The mixture was stirred at -30°C to -20°C for 1 h. The reaction was quenched by adding water (20 mL) at -10°C followed by 1 g of silica gel. The mixture was allowed to warm up to R.T. and stirred for 1 h. The mixture was filtered through celite and

CH_2Cl_2 was removed under reduced pressure. The residue was dissolved in 10 mL acetone and 5 mL water and was stirred with 0.3N HCl (0.4 mL) at R.T. for 30 min. After work up with ethyl acetate and column chromatography purification, hydroxyionone **120** – **123** (0.73 g, 3.5 mmol, 90%) was shown by HPLC (eluent A) and ^{1}H NMR to consist of a mixture of (3,6)-*trans*-3-hydroxy-α-ionone (34%) and (3,6)-*cis*-3-hydroxy-α-ionone (66%).

Reduction of (±)-3-Keto-α-Ionone Ketal (124) with Sodium Tri-*sec*-Butylborohydride (N-Selectride™). A solution of (±)-3-keto-α-ionone ketal (**124**) (50 mg, 0.20 mmol) in TBME (5 mL) was cooled down to -20°C under N_2 and a 1M solution of N-Selectride™ in THF (0.52 mL, 0.52 mmol) diluted with TBME (1 mL) was added by a gas-tight syringe. The mixture was stirred at this temperature for 0.5 h and the product was worked up and deprotected as described in the general procedure to give **120** – **123** (33.3 mg, 0.16 mmol; 80%). This was shown by HPLC (Eluent A) and NMR to consist of a mixture of (3,6)-*trans*-3-hydroxy-α-ionone (41%) and (3,6)-*cis*-3-hydroxy-α-ionone (59%).

Reduction of (±)-3-Keto-α-Ionone Ketal (124) with Potassium Tri-*sec*-Butylborohydride (K-Selectride™). A solution of (±)-3-keto-α-ionone ketal (**124**) (0.445 g, 1.78 mmol) in TBME (5 mL) was cooled down to -30°C under N_2 and a 1M solution of K-Selectride™ in THF (2.5 mL, 2.5 mmol) was added with an air-tight syringe in 15 min. The mixture was stirred at -30°C for 1 h and was then treated with 1.5 mL of 3 N NaOH followed by 1.5 mL of 30% H_2O_2. After stirring at R.T. for 30

min, the product was extracted with TBME (10 mL) and washed twice with water, dried over Na_2SO_4, and evaporated to dryness to give ketal **120** – **123** as a pale yellow oil. After deprotection with 0.3N HCl and purification by chromatography, the product was shown by HPLC (Eluent A) and ^{1}H NMR to consist of a mixture of (3,6)-*trans*-3-hydroxy-α-ionone (55%) and (3,6)-*cis*-3-hydroxy-α-ionone (45%) (0.315 g, 1.51 mmol, 85%).

Base-Catalyzed Isomerization of 3-Hydroxy-α-Ionone (120 – 123) to (±)-3-Hydroxy-β-Ionone (42). A solution of 3-hydroxy-α-ionone (**120** – **123**) (1.93g, 9.27 mmol) in THF (7 mL) was treated with 0.5 mL of a solution of KOH in methanol (10% wt./vol) under N_2. The mixture was heated to 50°C for 1 h and the product was partitioned between water and ethyl acetate. The organic layer was washed with water (2 X 100 mL), dried with Na_2SO_4, and evaporated to dryness to give 1.8 g of a yellow oil. The product was purified by column chromatography (hexane: ethyl acetate, from 98:2 to 90:10) to give a yellow oil which was identified as (±)-3-hydroxy-β-ionone (**42**) (1.26 g, 6.03 mmol; 65%). The spectroscopic data of **42** were identical with those of a standard sample of this ionone obtained by oxidative degradation of lutein described earlier.

Enzyme-Mediated Acylation of (±)-3-Hydroxy-β-Ionone (42) with Lipase PS (*pseudomonas cepacia*). To a solution of (±)-3-hydroxy-β-ionone (**42**) (3.3 g, 16 mmol) in 25 mL of ethyl acetate was added 5.0 g of immobilized lipase PS (*pseudomonas cepacia*) and vinyl acetate (1.0 mL, 0.93 g, 10 mmol). The mixture was

stirred at R.T. under N_2 and the course of the enzymatic acylation was monitored by chiral HPLC (Eluent B, hexane:isobutanol = 9/1). After 20 h the enzyme was filtered through celite and the filtrate was evaporated to dryness to give a yellow oil (4.0 g). Column chromatography (hexane: ethyl acetate, 98:2 to 80:20 of the product gave two major fractions.

The first fraction was identified from its ^{1}H NMR and UV spectrum as (*3R*)-3-acetoxy-β-ionone (**129**) (2.0 g, 8.0 mmol).

(*3R*)-3-Acetoxy-β-ionone (129): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.08 (s, 3H), 1.12 (s, 3H), 1.23 (s, 1H), 1.57 (t, *J* = 12.2, 1H), 1.74 (s, 3H), 1.77 (dd, *J* = 12.2, 3.5, 1H), 2.02 (s, 3H), 2.12 (dd, *J* = 17.1, 9.3, 1H), 2.27 (s, 3H), 2.47 (dd, *J* = 17.1, 6.0, 1H), 5.02 (m, 1H), 6.09 (d, *J* = 16.4, 1H), 7.17 (d, *J* = 16.4, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.3, 27.3, 28.3, 29.7, 36.3, 38.6, 43.9, 67.6, 131.3, 132.5, 135.6, 141.8, 170.6, 198.3. UV λ_{max} = 288 nm (ethanol). HRMS (EI) m/z calculated for $C_{15}H_{22}O_3$ 250.1569, found 250.1576. CD: 306 nm (-3.01 mdeg), 271 nm (+2.24 mdeg).

This fraction was dissolved in CH_2Cl_2 (30 mL) and stirred with KOH/MeOH (5.5 mL, 10% wt/v) for 2 hours at 0°C. The product was treated with 23 mL of 0.3N HCl to bring the pH to 5. The organic layer was sequentially washed with a saturated solution of $NaHCO_3$ (100 mL) and water (100 mL), and dried over Na_2SO_4. After solvent evaporation, 1.6 g of a yellow oil (7.7 mmol) was obtained which was identified by chiral HPLC (Eluent B, Appendix I) as (*3R*)-3-hydroxy-β-ionone (***3R*-42**) (96% *ee*). A small sample of this ionone was fully characterized from its UV, CD, ^{1}H- and ^{13}C-NMR, and mass spectra.

The second fraction (1.7 g, 8.2 mmol) was identified by chiral HPLC (Eluent B)

as (*3S*)-3-hydroxy-β-ionone (***3S*-42**) (96% *ee*). Similarly, this hydroxyionones was fully characterized from its UV, CD: 311 nm (3.20 mdeg), 273 nm (-3.59 mdeg), ^{1}H- and ^{13}C- NMR, and mass spectra.

The absolute configuration of hydroxyionones (***3R*)-42** and (***3S*)-42** was determined by comparison of their CD spectra with that of (*3R*)-3-hydroxy-β-ionone which was prepared by oxidative cleavage of naturally occurring (*3R,3'R,6'R*)-lutein.

Preparation of (*3R*)-3-Hydroxy-(β-Ionylideneethyl)triphenylphosphonium Chloride (16) From (*3R*)-3-Hydroxy-β-Ionone [(*3R*)-42] *via* (*3R*)-3-Hydroxy-Vinyl-β-Ionol [(*3R*)-43]. A solution of (*3R*)-3-hydroxy-β-ionone [(***3R*)-42**] (0.85 g, 4.1 mmol) in toluene (15 mL) was cooled down to -20°C under argon. A 1M solution of vinyl magnesium bromide (10 mL, 10 mmol) was added dropwise in 30 min and the mixture was stirred at this temperature for 1 h. The reaction was quenched with addition of 10 mL saturated ammonium chloride solution at -20°C and stirred at R.T. for 10 min. The product was partitioned between water (100 mL) and ethyl acetate (50 mL). The organic layer was washed with water (100 mL), dried over Na_2SO_4, and evaporated to dryness. The crude product (0.78 g), (*3R*)-3-hydroxy-vinyl-β-ionol [(***3R*)-43**], was dissolved in 5 mL MeOH and directly used without purification in the next step for the preparation of the Wittig salt (***3R*)-16**.

Triphenylphosphine hydrochloride was prepared fresh by adding 0.44 mL of concentrated HCl to triphenylphosphine (1.3 g, 4.9 mmol) in 5 mL methanol at 0°C. The salt was stirred at R.T. for 20 min and was treated with a solution of crude (*3R*)-3-hydroxy-vinyl-β-ionol [(***3R*)-43**] (0.78 g) in MeOH (5 mL) by dropwise addition in

5 min at 0°C. The reaction was kept at 0°C for 1 h and was allowed to stir to R.T. overnight. The product was partitioned between hexane (50 mL) and methanol:water = 1:1 (50 mL). The aqueous layer was washed with hexane (3 X 50 mL) to remove the excess triphenylphosphine and the aqueous layer was extracted with CH_2Cl_2 (3 X 50 mL). The combined CH_2Cl_2 layer was washed with water (100 mL), dried over Na_2SO_4, and evaporated to dryness to give 1.4 g crude product that was crystallized from 1,2-dichloroethane (11 mL) and ethyl acetate (25 mL) at -20°C. The crystals were washed with ethyl acetate and hexane and dried under high vacuum to give (*3R*)-3-hydroxy-(β-ionylideneethyl)triphenyl-phosphonium chloride [(***3R***)-**16**] (1.27 g, 2.46 mmol; 60%) as a grayish powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.95 (s, 3H), 0.97 (s, 3H), 1.34 (d, J = 4.2, 3H), 1.42 (t, J = 12.1, 1H), 1.60 (s, 3H), 1.73 (dd, J = 12.1, 3.47, 1H), 1.99 (dd, J = 16.8, 9.5, 1H), 2.16 (s, 1H), 2.32 (dd, J = 16.8, 4.9, 1H), 3.94 (m, 1H), 4.95 (m, 1H), 5.34 (dd, J = 14.3, 6.9, 1H), 5.90 (s, 2H), 7.66 (m, 6H), 7.77 (m, 3H), 7.87 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.7, 21.4, 24.4, 24.9, 28.5, 30.1, 36.8, 42.3, 48.0, 64.6, 113.3, 113.4, 117.9, 118.7, 126.8, 127.6, 127.7, 130.1, 130.3, 133.8, 133.9, 134.8, 134.9, 136.2, 136.3, 136.8, 143.5, 143.6. UV λ_{max} = 268 nm (ethanol). CD: 279 nm (-4.07 mdeg). The NMR and CD were identical with standard sample of (***3R***)-**16** obtained from the DSM Nutritional Products (Basel, Switzerland).

Preparation of (*3S*)-3-Hydroxy-(β-Ionylideneethyl)triphenylphosphonium Chloride [(*3S*)-16] From (*3S*)-3-Hydroxy-β-Ionone [(*3S*)-42] *via* (*3S*)-3-Hydroxy-Vinyl-β-Ionol [(*3R*)-43]. Employing the same procedure described above, (*3S*)-3-hydroxy-β-ionone (***3S***)-**42** (0.85 g, 4.1 mmol) was transformed into (*3S*)-3-hydroxy-

(β-ionylideneethyl)triphenyl-phosphonium chloride [(***3S***)-**16**] (1.2 g, 2.3 mmol, 55%). The ^{1}H and ^{13}C-NMR spectra of (***3S***)-**16** were identical with those of (***3R***)-**16**. UV λ_{max} = 268 nm (ethanol). CD: 281 nm (+6.43 mdeg).

Synthesis of (*3R,3′R*)-Zeaxanthin (5). A mixture of (*3R*)-3-hydroxy-(β-ionylideneethyl)triphenylphosphonium chloride [(***3R***)-**16**] (0.30 g, 0.58 mmol), C_{10}-dialdehyde **17** (45 mg, 0.28 mmol), 1,2-epoxybutane (0.5 mL) in ethanol (5 mL) was refluxed under N_2 and the course of the reaction was monitored by HPLC (eluent A, Appendix I). After 22 h, HPLC showed the completion of the reaction. The product was filtered and the solids were washed with ethanol. Crystallization from CH_2Cl_2 and hexane gave a red solid that was identified from its NMR, CD, UV-Vis, and MS spectra as (*3R,3′R*)-zeaxanthin (**5**) (68 mg, 0.12 mmol, 43%). The product was shown by chiral HPLC (Eluent L, Appendix I) to have an enantiomeric excess (*ee*) of 98%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.08 (s, 12H), 1.38 (s, 2H), 1.49 (t, J = 12.0, 2H), 1.75 (s, 6H), 1.78 (dd, J = 12.1, 3.5, 2H), 1.98 (s, 12H), 2.06 (dd, J = 16.9, 9.7, 2H), 2.40 (dd, J = 16.9, 5.4, 2H), 4.01 (m, 2H), 6.08-6.20 (m, 6H), 6.26 (d, J = 7.6, 2H), 6.37 (d, J = 15.0, 2H), 6.60-6.70 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.7, 19.3, 21.6, 28.7, 30.3, 37.1, 42.5, 48.5, 65.1, 124.9, 125.5, 126.2, 130.1, 131.3, 132.7, 135.6, 136.6, 137.6, 137.9, 138.5. UV/Vis λ_{max} = 450 nm (ethanol). HRMS (ESI) m/z calculated for $C_{40}H_{56}O_2$ 568.4280, found 568.4277. CD: 342 nm (+1.80 mdeg), 284 nm (-8.39 mdeg), 245 nm (+5.17 mdeg), 222 nm (-5.86 mdeg).

Synthesis of (*3S,3'S*)-Zeaxanthin (6). A mixture of (*3S*)-3-hydroxy-(β-ionylideneethyl)triphenylphosphonium chloride [(***3S***)-**16**] (0.30 g, 0.58 mmol), C_{10}-dialdehyde **17** (45 mg, 0.28 mmol), 1,2-epoxybutane (0.5 mL) in ethanol (5 mL) was refluxed under N_2 and the course of the reaction was monitored by HPLC (eluent A). After 22 h, HPLC showed the completion of the reaction. The product was filtered and the solids were washed with ethanol. Crystallization from CH_2Cl_2 and hexane gave a red solid that was identified from its NMR, CD, UV-Vis, and MS spectra as (*3S,3'S*)-zeaxanthin (**6**) (63 mg, 0.11 mmol, 39%); according to chiral HPLC (Eluent L, Appendix I) this product was obtained in 98% *ee*. The ^{1}H- and ^{13}C-NMR were identical with **5**. UV/Vis λ_{max} = 450 nm (ethanol). HRMS (ESI) calculated for $C_{40}H_{56}O_2$ 568.4280, found 568.4279 CD: 342 nm (-1.88 mdeg), 284 nm (+8.48 mdeg), 246 nm (-5.38 mdeg), 222 nm (+5.87 mdeg).

Synthesis of (*3R*)-β-Cryptoxanthin (134). A mixture of β-apo-12'-carotenal (**134**) (0.16 g, 0.45 mmol), (*3R*)-3-hydroxy-(β-ionylideneethyl)triphenylphosphonium chloride [(***3R***)-**16**] (0.27 g, 0.52 mmol), and 1,2-epoxybutane (0.39 mL) in ethanol (5 mL) was refluxed under N_2. After 6 h, the product was filtered and the solids were washed with ethanol. Crystallization from CH_2Cl_2 and hexane gave a red solid that was identified as (*3R*)-β-cryptoxanthin (**135**) (0.18 g, 0.32 mmol; 70%), m.p. 134-136°C. The product was shown by chiral HPLC (Eluent M, Appendix I) to have an optical purity of 98% (*ee*).

135: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.04 (s, 6H), 1.08 (s, 6H), 1.47 (m, 2H), 1.61-1.65 (m, 2H), 1.72(s, 3H), 1.75 (s, 3H), 1.78 (dd, J = 12.1, 3.5, 2H), 1.98 (s, 12H),

2.02 (t, *J* = 6.2, 1H), 2.07 (d, *J* = 9.8, 1H), 2.40 (dd, *J* = 16.8, 5.3, 1H), 4.01 (m, 1H), 6.08-6.21 (m, 6H), 6.26 (d, *J* = 6.3, 2H), 6.37 (dd, *J* = 14.9, 3.6, 2H), 6.60-6.70 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.8, 19.3, 21.6, 21.8, 28.7, 29.0, 30.3, 33.1, 34.3, 37.1, 39.7, 42.6, 48.5, 65.1, 124.9, 125.1, 125.5, 126.2, 126.7, 129.4, 129.9, 130.1, 130.8, 131.3, 132.4, 132.7, 135.6, 136.1, 136.4, 136.6, 137.2, 137.6, 137.8, 137.9, 138.5. UV/Vis λ_{max} = 450 nm (ethanol). MS (FAB^+) calculated for $C_{40}H_{55}O$ $[M+H]^+$ 552.4330, found 552.4000. CD: 342 nm (+0.88 mdeg), 286 nm (-4.13 mdeg), 248 nm (+2.65 mdeg), 222 nm (-2.80 mdeg).

Synthesis of (*3S*)-β-Cryptoxanthin (136). β-Apo-12'-carotenal (**134**) (0.16 g, 0.45 mmol) was allowed to react with (*3S*)-3-hydroxy-(β-ionylideneethyl)-triphenylphosphonium chloride [(***3S***)-**16**] (0.27 g, 0.52 mmol) in the presence of 1,2-epoxybutane (0.39 mL) under reflux in ethanol (5 mL). After 6 h, the product was worked up and crystallized as described in the previous experiment to afford (*3S*)-β-cryptoxanthin (**136**) (0.18 g, 0.32 mmol; 70%). This was shown by chiral HPLC (Eluent M) to have an optical purity of 98% (*ee*). The ^{1}H-, ^{13}C-NMR, UV/Vis, and MS were identical to those of **135**. CD: 342 nm (-1.08 mdeg), 284 nm (+4.12 mdeg), 248 nm (-2.78 mdeg), 224 nm (+2.85 mdeg).

Synthesis of C_{20}-3-Hydroxynitriles (93+94) from 3-Hydroxy-α-Ionylidene-acetaldehydes (73+74). To a solution of 3-methyl-4-oxobut-2-enenitrile (31 mg, 0.090 mmol) in 3 mL TBME was added butyl lithium (1.6 M in hexane, 0.96 mL, 1.5 mmol) at 0°C under N_2. The ice bath was removed and the reaction mixture was

stirred for 0.5 h at R.T. The mixture was cooled down to 0°C and a recemic mixture of hydroxyaldehydes **73**+**74** (0.20 g, 0.86 mmol) in 5 mL TBME was added dropwise at 0^{o}C. After stirring for 1 h at R.T., the product was worked up by adding saturated NH_4Cl (10 mL) and EtOAc (15 mL). The EtOAc layer was dried over Na_2SO_4 and evaporated to dryness. After purification by column chromatography (hexane: ethyl acetate from 95:5 to 85:15), a small portion of the product (0.20 g, 0.69 mmol, 83%) was further purified by semipreparative HPLC (Eluent N, Appendix I) to yield (±)-3-hydroxy-α-ionylideneacetaldehydes (**93**+**94**).

93+94: ^{1}H NMR (400MHz, $CDCl_3$) δ 0.86 (s, 3H), 1.01 (s, 3H), 1.38 (dd, *J* = 13.3, 6.8, 1H), 1.63 (s, 3H), 1.84 (dd, *J* = 13.3, 6.0, 1H), 1.96 (d, *J* = 0.8, 3H), 2.22 (d, *J* = 1.2, 3H), 2.43 (d, *J* = 9.9, 1H), 4.26 (br, 1H), 5.20 (s, 1H), 5.57 (s, 1H), 5.6 (dd, *J* = 15.3, 9.7, 1H), 6.11 (d, *J* = 11.3, 1H), 6.16 (d, *J* = 15.3, 1H), 6.30 (d, *J* = 15.1, 1H), 6.92 (dd, *J* = 15.1, 11.1, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.3, 16.6, 22.8, 24.3, 29.5, 34.0, 44.5, 54.9, 65.8, 96.8, 118.1, 124.8, 128.8, 131.7, 131.9, 132.3, 137.0, 137.4, 140.3, 156.9. UV λ_{max} = 338 nm (hexane); HRMS (ESI^{+}) calculated for $C_{20}H_{27}NO$ $[M+Li]^{+}$ 304.2252, found 304.2250.

Reduction of (±)-C_{20}-3-Hydroxynitrile (93+94) to (±)-3-Hydroxy-α-Retinal (89+90). A solution of (±)-C_{20}-3-hydroxynitriles (**93**+**94**) (0.15 g, 0.50 mmol) in 5 mL CH_2Cl_2 was cooled down to -40°C under N_2 and a 1M solution of DIBAL-H in CH_2Cl_2 (1.5 mL, 1.5 mmol) was added dropwise in one hour. After the addition was completed, the reaction mixture was allowed to stir at -30°C for 1 h. The mixture was very slowly treated with a homogeneous mixture of 3 g of water absorbed on n-silica

(0.3 g of water/g of silica) at a rate that the temperature remained below -10°C [caution: the addition of silica/water results in rapid elevation of the temperature]. After the addition was completed, the reaction mixture was allowed to stir at 0°C for 1 h. Na_2SO_4 (3 g) was added and the solids were filtered off and washed with CH_2Cl_2 (20 mL). The organic layer was washed with water, dried over Na_2SO_4, and evaporated to dryness to give a yellow oil. Column chromatography (hexane:ethyl acetate, 98:2 to 88:12) of the product gave **89**+**90** (94 mg, 0.31 mmol; 62%).

89+**90:** ^{1}H NMR (400MHz, $CDCl_3$) δ 0.86 (s, 3H), 1.01 (s, 3H), 1.39 (dd, *J* = 13.1, 6.8, 1H), 1.63 (s, 3H), 1.85 (dd, *J* = 13.1, 5.9, 1H), 1.98 (s, 3H), 2.33 (d, *J* = 1.0, 3H), 2.44 (d, *J* = 10.2, 1H), 4.27 (br, 1H), 5.58 (s, 1H), 5.61 (dd, *J* = 15.4, 10.2, 1H), 5.98 (d, *J* = 8.2, 1H), 6.17 (d, *J* = 15.4, 1H), 6.18 (d, *J* = 11.0, 1H), 6.38 (d, *J* = 15.1, 1H), 7.11 (dd, *J* = 15.1, 11.0, 1H), 10.11 (d, *J* = 8.2, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.1, 13.3, 22.8, 24.3, 29.5, 34.0, 44.5, 55.0, 65.8, 124.8, 129.2, 129.4, 131.9, 132.2, 134.9, 137.1, 137.4, 140.2, 154.6, 184.5, 191.1. UV λ_{max} = 360 nm nm (hexane). HRMS (ESI^+) calculated for $C_{20}H_{28}O_2$ $[M+H]^+$ 301.2167, found 301.1907.

Preparation of (±)-Vinyl-α-Ionol (116). A solution of vinylmagnesium bromide (1M, 0.1 mol) in THF (100 mL) was added dropwise to a solution of (±)-α-ionone (18 g, 94 mmol) in THF (20 mL) at –20°C under N_2. The reaction mixture was stirred for 1 h at 0°C and 30 minutes at R.T., and subsequently was cooled down to –20°C and treated with 70 mL of saturated ammonium chloride solution. The product was extracted with TBME (2 X 100 mL), dried over Na_2SO_4, and evaporated to dryness to give a colorless oil. The crude product was purified by fractional distillation (b.p. =

94°C at 0.5mm/Hg) to yield (±)-vinyl-α-ionol (**116**) (17.7 g, 80.4 mmol; 86%): R_f = 0.58 (hexane/acetone, 9/1); the NMR data was consistent with that of literature.[96]

Bleach Oxidation of (±)-Vinyl-α-Ionol (116) to (±)-3-Keto-Vinyl-α-Ionol (115). (±)-Vinyl-α-ionol (**116**) (3.0 g, 14 mmol) was transferred into a 1 L three-necked flask using ethyl acetate (13.4 mL, 12.0 g, 136 mmol). K_2CO_3 (0.19 g, 1.4 mmol) was added and the mixture was cooled down in an ice-salt bath to 0°C under N_2. A 70% solution of TBHP in water (11.7 mL, 10.5 g, 70% ≈ 7.40 g, 81.7 mmol) was added dropwise to the mixture under N_2 at 0°C in 10 mins. Household bleach containing 5.25% NaOCl (38.6 g, 2.03 g NaOCl, 27.2 mmol) was then added over a period of 7 h at -5 to 0°C. After the addition was completed, the reaction mixture was stirred at 0°C for additional 10 h. The product was extracted with hexane (200 mL) and the organic layer was separated. The water layer was washed with hexane (2 X 100 mL) and the combined organic layer was washed with water (3 X 200 mL), dried over Na_2SO_4, and evaporated to give 2.9 g of a yellow oil. The crude product was purified by column chromatography (hexane:acetone, from 98:2 to 90:10) to yield a mixture of 3-keto-vinyl-α-ionol (**115**) (2.51 g, 10.7 mmol; 79%) as a colorless oil. The ^{1}H NMR data of **115** was consistent with that of literature.[96]

$Rh_2(cap)_4$ Catalyzed Oxidation of (±)-Vinyl-α-Ionol (116) to (±)-3-Keto-Vinyl-α-Ionol (115). To a solution of (±)-vinyl-α-ionol (**116**) (1.0 g, 4.5 mmol) in 10 mL of CH_2Cl_2 was added K_2CO_3 (0.30 g, 2.3 mmol) and $Rh_2(cap)_4$ (2 mg, 0.005 mmol); this was followed by the addition of *tert*-butylhydroperoxide (6.3 M in decane, 3.6 mL, 23

mmol). The reaction mixture was stirred at 0°C for 2 hours and then at R.T. for 22 h. The product was filtered through celite and the filtrate was washed with water (2 X 30 mL), dried over Na_2SO_4, and evaporated to dryness to give a colorless oil. Purification by column chromatography (hexane/acetone, 95/5) afforded 0.6 g (60%) of (±)-3-keto-vinyl-α-ionol (**115**): R_f = 0.47 (hexane/acetone, 8/2); ^{1}H NMR data of **115** was consistent with that of literature.[90]

Pd(II)-Mediated Oxidation of (±)-Vinyl-α-Ionol (116) to (±)-3-Keto-Vinyl-α-Ionol (115). To a solution of (±)-vinyl-α-ionol **116** (30.0 g, 136 mmol) in 50 mL of hexane was added K_2CO_3 (4.85 g, 35.1 mmol) and Pd/C (10% on activated carbon, 3.75 g, 3.52 mmol). The mixture was cooled down to 0°C in an ice bath and *tert*-butylhydroperoxide (70% in water, 65.3 mL, 681 mmol) was added dropwise under N_2. The reaction mixture was stirred at 0°C for 2 hours and at R.T. for 20 h and then filtered through celite. The filtrate was washed with water (3 X 100 mL) and the organic layer was dried over Na_2SO_4 and evaporated to dryness to give a colorless oil. Purification by column chromatography (hexane/acetone, 95/5) afforded 14.2 g (44.5%) of (±)-3-keto-vinyl-α-ionol (**115**). The structure of this product was confirmed from its ^{1}H NMR spectrum.

Preparation of (*E*)-Ethyl-3-Formyl-2-Butenoate (113). To a flask containing sodium hydride (5.6 g, 0.14 mol) and 30 mL of TBME was added triethyl phosphonoacetate (22 g, 0.10 mol) in 30 mL TBME at 0°C. The reaction mixture was stirred for 1 h at R.T. and was then treated with pyruvaldehyde dimethylacetal (14 g,

0.12 mmol) in 30 mL TBME at 10°C. The mixture was stirred for 24 hours at R.T. and the product was washed with water. The aqueous layer was washed with TBME (3 X 30 mL) and the combined organic layer was washed with water (2 X 30 mL), dried over Na_2SO_4, and evaporated to dryness. The crude product (17g) was dissolved in CH_2Cl_2 (30 mL) and then subjected to cis-trans isomerization by treatment with 30 mL HCl (3N) at R.T. for 4 hours. The *all-trans* product was then purified by column chromatography (hexane/acetone, 98/2) to yield **113** as a colorless liquid (9.0 g, 76 mmol; 64%, *E/Z* = 49/1).

(*E*)-Ethyl-3-formyl-2-butenoate (113): ^{1}H NMR (400MHz, $CDCl_3$): δ 1.26 (t, J = 7.0, 3H), 2.07 (d, J = 1.5, 3H), 4.24 (q, J = 14.3, 7.0, 2H), 6.46 (dd, J = 3.02, 1.5, 1H), 9.47 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.0, 60.9, 104.9, 135.4, 165.3, 194.3.

(*Z*)-Ethyl-3-formyl-2-butenoate (113): ^{1}H NMR (400MHz, $CDCl_3$): δ 1.24 (t, J = 5.3, 3H), 1.90 (d, J = 1.8, 3H), 4.13 (q, J = 7.0, 2H), 5.98 (s, 1H), 10.6 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 10.5, 53.1, 104.0, 118.2, 150.2, 203.8.

Synthesis of (±)-3-Keto-α-Retinoic Acid Ethyl Esters (17) from (±)-3-Keto-Vinyl-α-Ionol (115). To a solution of (±)-3-keto-vinyl-α-ionol (**115**) (2.74 g, 11.7 mmol) in 15 mL of ethanol was added triphenylphosphine hydrobromide (4.0 g, 12 mmol). The mixture was stirred at R.T. for 2 hours and then partitioned between water (50 mL) and hexane (50 mL). The organic layer was removed and the aqueous layer was washed with hexane (2 X 30 mL). The water layer was then extracted with CH_2Cl_2 (2 X 30 mL) and the combined organic layer was washed with water (2 X 50 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was transferred into a

three-neck flask equipped with an addition funnel and a low temperature thermometer. A solution of (*E*)-ethyl-3-formyl-2-butenoate (**113**) (1.58 g, 11.1 mmol) in ethanol (20mL) was added and the mixture was cooled to –20°C. A freshly prepared solution of NaOEt (from 0.30 g of Na, 13 mmol) in EtOH (10 mL) was added dropwise to the reaction mixture and the course of the reaction was monitored by normal phase HPLC (Eluent O, Appendix I). After stirring at –10°C for 4 h, the product was partitioned between hexane (30 mL) and water (30 mL). The organic layer was removed and the aqueous layer was extracted with hexane (2×30 mL). The combined organic layer was washed with brine (25 mL) and water (25 mL), dried over Na_2SO_4, and evaporated to dryness to give 5.9 g of a yellow oil. This was shown by HPLC (Eluent O, Appendix I) to consist of three major fractions. The crude product was quickly passed through a silica gel column using hexane/acetone (98/2) to yield a mixture of (*E*/Z)-(±)-3-keto-α-retinoic acid ethyl esters (**112**) (2.6 g, 65%). A small portion of this product was subjected to semipreparative HPLC (Eluent O, Appendix I) to separate three fractions that in the order of chromatographic elution were identified as: (11*Z*)-3-keto-α-retinoic acid ethyl ester (**11*Z*-112**), (*all-E*)-3-keto-α-retinoic acid ethyl ester (***all-E*-112**), and (9*Z*)-3-keto-α-retinoic acid ethyl ester (**9*Z*-112**) (11*Z*: *all-E*: 9*Z* = 1.6:4.2:1).

(11*Z*)-3-Keto-α-retinoic acid ethyl ester (11*Z*-112): ^{1}H NMR (400MHz, $CDCl_3$) δ 0.97 (s, 3H), 1.05 (s, 3H), 1.28 (t, *J* = 7.3, 3H), 1.90 (s, 3H), 1.91 (d, *J* = 1.3, 3H), 2.10 (d, *J* = 16.9, 1H), 2.33 (d, *J* = 1.3, 3H), 2.37 (d, *J* = 16.9, 1H), 2.61 (d, *J* = 9.4, 1H), 4.19 (q, *J* = 14.2, 7.3, 2H), 5.62 (dd, *J* = 15.4, 9.4, 1H), 5.84 (s, 1H), 5.91 (s, 1H), 5.95 (d, *J* = 10.7, 1H), 6.25 (d, *J* = 15.4, 1H), 6.50 (dd, *J* = 12.3, 10.7, 1H), 6.52 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.6, 14.3, 19.2, 23.6, 27.4, 27.9, 36.5, 47.6, 56.4,

59.8, 119.6, 125.7, 126.9, 127.4, 128.2, 132.6, 138.1, 138.4, 153.0, 162.0, 166.8, 199.1. UV λ_{max} = 330 nm (hexane). MS (DEI^+) m/z calculated for $C_{32}H_{30}O_3$ 342.4718, found 342.2002**.**

(*all-E*)-3-Keto-α-retinoic acid ethyl ester (*all-E*-112): ^{1}H NMR (400MHz, $CDCl_3$) δ 0.96 (s, 3H), 1.04 (s, 3H), 1.28 (t, *J* = 7.0, 3H), 1.89 (d, *J* = 1.26, 3H), 1.93 (d, *J* = 1.0, 3H), 2.09 (d, *J* = 17.8, 1H), 2.33 (d, *J* = 1.25, 3H), 2.35 (d, *J* = 17.8., 1H), 2.61 (d, *J* = 9.2, 1H), 4.15 (q, *J* = 7.0, 14.3, 2H), 5.64 (dd, *J* = 15.5, 9.2, 1H), 5.78 (s, 1H), 5.91 (m, 1H), 6.16 (d, *J* = 11.4, 1H), 6.22 (d, *J* = 15.5, 1H), 6.30 (d, *J* = 15.1, 1H), 6.93 (dd, *J* = 15.1, 11.4, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.1, 13.7, 14.3, 23.6, 26.5, 27.3, 27.9, 36.5, 47.5, 56.3, 59.6, 119.3, 125.7, 127.5, 130.2, 130.7, 136.3, 137.7, 138.0, 152.2, 161.8, 167.0, 199.1. UV λ_{max} = 336 nm (hexane). MS was same as that of (*9Z*)-**112**.

(9Z)-3-Keto-α-retinoic acid ethyl ester (9Z-112): ^{1}H NMR (400MHz, $CDCl_3$) δ 0.99 (s, 3H), 1.08 (s, 3H), 1.30 (t, *J* = 7.0, 3H), 1.94 (d, *J* = 1.3, 3H), 1.94 (s, 3H), 2.14 (d, *J* = 17.1, 1H), 2.37 (d, *J* = 1.1, 3H), 2.39 (d, *J* = 17.1, 1H), 2.69 (d, *J* = 9.8, 1H), 4.18 (q, *J* = 14.3, 7.0, 2H), 5.64 (dd, *J* = 15.3, 9.8, 1H), 5.8 (s, 1H), 5.93 (m, 1H), 6.08 (d, *J* = 11.4, 1H), 6.25 (d, *J* = 15.0, 1H), 6.77 (d, *J* = 15.3, 1H), 7.04 (dd, *J* = 15.0, 11.4, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.9, 14.3, 21.1, 23.7, 27.4, 27.9, 36.5, 47.7, 56.8, 59.7, 119.3, 125.7, 128.9, 129.2, 129.5, 130.2, 135.6, 136.5, 152.2, 161.8, 167.0, 199.0. UV λ_{max} = 332 nm (hexane). MS was same as that of (*9Z*)-**112**.

***Z/E*-Isomerization of (±)-3-Keto-α-Retinoic Acid Ethyl Ester (112) with $Pd(OAc)_2$.** To the mixture of (±)-3-keto-α-retinoic acid ethyl esters (**112**) (11*Z*:*all*-

E:9*Z* = 1.6:4.2:1) (1.1 g, 3.2 mmol) was added 5 mg of $Pd(OAc)_2$ in 30 mL hexane. The mixture was refluxed for 24 h and the progress of isomerization was monitored by normal phase HPLC (System 3). The product was filtered through celite and the solvent was evaporated to dryness. The residue was examined by HPLC (Eluent O, Appendix I) and was shown to consist of (*all-E*)-3-keto-α-retinoic acid ethyl ester [(*all-E*)-**112**, 84%] and (*9Z*)-3-keto-α-retinoic acid ethyl ester [(*9Z*)-**112**,16%].

Reduction of 3-Keto-α-Retinoic Acid Ethyl Ester (112) to 3-Hydroxy-α-Retinoic Acid Ethyl Esters (108 – 111) with Sodium bis(2-Methoxyethoxy)aluminum Hydride (Red-Al™). A solution of (*all-E*)-(±)-3-keto-α-retinoic acid ethyl ester [(*all-E*)-**112**] (0.31 g, 0.90 mmol) purified by semipreparative HPLC (Eluent O, Appendix I) in toluene (10 mL) was cooled down to -10°C under nitrogen and a solution of Red-Al (1.0 mL of 1M in toluene, 1.0 mmol) was added. The course of the reaction was monitored by HPLC (Eluent O, Appendix I). The mixture was stirred at -10°C for 2 h and the reaction was quenched by adding water (5 mL). The product was diluted with TBME (5 mL) and washed sequentially with brine and water. The organic layer was dried over Na_2SO_4 and evaporated to afford 3-hydroxy-α-retinoic acid ethyl esters (**108** – **111**) in nearly quantitative yield (0.29 g, 0.85 mmol, 95%). Two pairs of diastereomeric hydroxyesters were separated by semipreparative HPLC and identified as a mixture of **108+109** (50%) and **110+111** (50%).

(3,6-*trans*)-3-Hydroxy-α-retinoic acid ethyl ester (108+109): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86 (s, 3H), 1.00 (s, 3H), 1.29 (t, J = 7.0, 2H), 1.39 (dd, J = 13.1, 6.8,

1H), 1.48 (br, 1H), 1.62 (s, 3H), 1.84 (dd, *J* = 13.1, 5.8, 1H), 1.94 (s, 3H), 2.34 (d, *J* = 1.3, 1H), 2.42 (d, *J* = 10.0, 1H), 4.15 (q, *J* = 7.0, 2H), 4.25 (br, 1H), 5.54 (dd, *J* = 15.5, 10.0, 1H), 5.56 (m, 1H), 5.78 (s, 1H), 6.13 (d, *J* = 11.3, 1H), 6.15 (d, *J* = 15.5, 1H), 6.29 (d, *J* = 15.0, 1H), 6.95 (dd, *J* = 15.0, 11.3, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.7, 13.2, 13.8, 14.3, 22.8, 24.2, 29.4, 34.2, 44.5, 54.9, 59.7, 65.8, 118.8, 124.7, 129.5, 130.6, 130.8, 135.5, 137.2, 137.5, 138.5, 138.6, 152.5, 167.1. UV λ_{max} = 338 nm (hexane). MS (ESI^+) calculated for $C_{22}H_{32}O_3$ $[M+H-H_2O]^+$ 327.2324, found 327.2001.

(3,6-*cis*)-3-Hydroxy-α-retinoic acid ethyl ester (110+111): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.85 (s, 3H), 0.95 (s, 3H), 1.27 (q, *J* = 5.3, 2H), 1.29 (t, *J* = 5.3, 3H), 1.39 (dd, *J* = 12.6, 9.8, 1H), 1.59 (m, 1H), 1.63 (m, 3H), 1.93 (s, 3H), 2.17 (d, *J* = 9.4, 1H), 2.34 (d, *J* = 1.01, 3H), 4.17 (q, *J* = 7.3, 2H), 4.23 (s, 1H), 5.46 (s, 1H), 5.64 (dd, *J* = 15.5, 9.4, 1H), 5.77 (s, 1H), 6.13 (d, *J* = 11.6, 1H), 6.14 (d, *J* = 15.5, 2H), 6.28 (d, *J* = 15.1, 1H), 6.95 (dd, *J* = 15.7, 11.6, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.2, 13.8, 14.3, 22.6, 27.0, 29.2, 34.7, 40.9, 55.0, 60.0, 66.7, 118.8, 124.7, 129.5, 130.7, 131.8, 135.5, 136.2, 137.6, 138.7, 152.5, 166.8. UV λ_{max} = 334 nm. MS (ESI^+) calculated for $C_{22}H_{32}O_3$ $[M+H-H_2O]^+$ 327.2324, found 327.3113.

Synthesis of C_{20}-3-Hydroxy-Retinals 89 – 92 from 3-Hydroxy-α-Retinoic Acid Ethyl Esters (108 – 111). To a flask containing 3-hydroxy-α-retinoic acid ethyl esters (**108** – **111**) (0.25 g, 0.73 mmol) in 15 mL THF was added $LiAlH_4$ (1 M in THF, 1.5 mL, 1.5 mmol) at -25°C. The mixture was allowed to warm up to -10°C and stirred for 2 hour. The reaction was monitored by HPLC (Eluent O, Appendix I) and the

product was worked up by adding saturated NH_4Cl (20 mL) and extracted with 30 mL EtOAc. The EtOAc layer was then dried over Na_2SO_4 and evaporated to dryness to obtain the crude product (0.3 g) as a pale yellow oil. Without purification, this diol was dissolved in 5 mL of CH_2Cl_2 and treated with MnO_2 (65 mg, 0.75 mmol) at R.T. After stirring for 2 h, HPLC showed the completion of the reaction. The MnO_2 was filtered off and the filtrate was evaporated to dryness. The crude product was then purified by column chromatography (hexane:ethyl acetate, 98:2 to 88:12) to afford **89** – **92** in 61% yield (0.13 g, 0.44 mmol). A small sample of this mixture was then subjected to semipreparative HPLC (Eluent N, Appendix I) to yield two fractions. The first fraction was identified from its ^1H and ^{13}C-NMR spectra as a racemic mixture of **89** and **90** prepared earlier. The second fraction was identified from its proton NMR as a racemic mixture of **91** and **92**.

91+**92:** ^1H NMR (400 MHz, $CDCl_3$) δ 0.87 (s, 3H), 0.96 (s, 3H), 1.40 (dd, J = 13.6, 9.8, 2H), 1.65 (t, J = 1.75, 3H), 1.68 (dd, J = 6.5, 1H), 1.97 (d, J = 1.0, 1H), 2.20 (d, J = 9.3, 1H), 2.33 (d, J = 1.0, 1H), 4.26 (m, 1H), 5.52 (s, 1H), 5.71 (dd, J = 15.4, 9.4, 1H), 5.98 (d, J = 8.0, 1H), 6.17 (d, J = 15.3, 1H), 6.18 (d, J = 11.5, 1H), 6.37 (d, J = 15.1, 1H), 7.11 (dd, J = 15.1, 11.5, 1H).

General Procedure for Base-Catalyzed Isomerization of 3-Hydroxy-α-Ionylideneacetonitriles (77 – 80) with KOH (Table 8, Entry 1-4).

Base-Catalyzed Isomerization of 3-Hydroxy-α-Ionylideneacetonitriles (77 – 80) (Table 8, Entry 3). A solution of 3-hydroxy-α-ionylideneacetonitriles (**77** – **80**) (71 mg, 0.31 mmol) in 15 mL ethylene glycol mono ether was treated with 0.3 mL of a

solution of KOH (5.3 mmol) in the same alcohol (5% wt./vol) under N_2. The mixture was heated at 120°C overnight and the product was partitioned between water and EtOAc. The organic layer was washed with water (2 X 100 mL), dried with Na_2SO_4, and evaporated to dryness to give 1.8 g of a yellow oil. The product was shown by HPLC (Eluent A, Appendix I) to consist of 35% of unreacted starting material **77** – **80** (**77**+**78** : **79**+**80** = 1.0 : 3.0) and 65% of a racemic mixture of 3-hydroxy-β-ionylideneacetonitriles (**102**+**103**). This isomerization was also carried out under similar conditions in three other solvents (ethanol, propanol, and ethylene glycol) and the results are summarized in Table 8 in the results and discussion section of this dissertation. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.07 (d, J = 2.3, 6H), 1.48 (t, J = 12.1, 1H), 1.66 (s, 1H), 1.71 (s, 3H), 1.78 (dd, J = 12.1, 3.6, 1H), 2.06 (dd, J = 17.2, 9.5, 1H), 2.20 (d, J = 1.0, 3H), 2.40 (dd, J = 17.2, 5.7, 1H), 4.00 (m, 1H), 5.18 (d, J = 0.8, 1H), 6.13 (d, J = 16.1, 1H), 6.50 (dd, J = 16.1, 0.8, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.5, 21.5, 28.6, 30.1, 37.0, 42.4, 48.1, 64.6, 96.9, 117.8, 129.2, 133.4, 134.7, 136.4, 156.9. UV λ_{max} = 298 nm (hexane). MS (ESI^+) calculated for $C_{15}H_{21}NO$ $[M+H]^+$ 232.1701, found 232.1861.

Base-Catalyzed Isomerization of 3-Hydroxy-α-Ionylideneacetonitriles (77 – 80) with KF-alumina (Table 8, Entry 5 and 6). A solution of 3-hydroxy-α-ionylideneacetonitriles (**77** – **80**) (71 mg, 0.31 mmol) in 5 mL DMF was treated with KF-Al_2O_3 (40% by wt, 90 mg ≈ 36 mg, 0.62 mmol) under N_2. The mixture was heated to 120°C for 29 hours and the product was partitioned between water and EtOAc. The organic layer was washed with water (2 X 100 mL), dried with Na_2SO_4, and

evaporated to dryness to give a yellow oil. The product was shown by HPLC (Eluent A, Appendix I) to consist of 36% of unreacted starting material **77** – **80** (**77**+**78** : **79**+**80** = 1.0 : 4.4) and 64% of a racemic mixture of 3-hydroxy-β-ionylideneacetonitriles (**102**+**103**). This isomerization was also carried out under similar conditions in ethylene glycol (Table 8, Entry 6) and the results are summarized in Table 8 in the results and discussion section of this dissertation.

Synthesis of 3-Hydroxy-Retinol (98) from (*3R*)-16. A solution of (*3R*)-3-hydroxy-(β-ionylideneethyl)triphenylphosphonium chloride [(***3R***)**-16**] (1.7 g, 3.3 mmol) and (*E*)-3-formyl-2-butenyl acetate (0.50 g, 4.3 mmol) in CH_2Cl_2 (10 mL) was cooled down to -20°C under N_2. A solution of NaOMe (0.10 g of Na, 4.3 mmol) in 3mL of MeOH was added in 20 minutes and the mixture was stirred at -10°C for 3.5 h. Dichloromethane (20 mL) was added, and the product was washed with water (3 X 10 mL). The organic layer was removed, dried over Na_2SO_4, and evaporated to dryness to give 2.3 g of a yellow oil. The crude product was purified by column chromatography (hexane:ethylacetate, from 95:5 to 88:12) to give **88** (0.80 g, 2.6 mmol, 80%) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.07 (s, 6H), 1.34 (s, 1H), 1.48 (t, *J* = 12.1, 1H), 1.74 (s, 3H), 1.77 (dd, *J* = 12.1, 5.9, 1H), 1.97 (s, 3H), 2.04 (dd, *J* = 16.9, 9.5, 1H), 2.34 (s, 3H), 2.39 (dd, *J* = 16.9, 5.9), 4.01 (m, 1H), 4.33 (d, *J* = 7.0, 2H), 6.10 (d, *J* = 11.0, 1H), 6.20 (d, *J* = 16.0, 2H), 6.33 (dd, J =15.0, 11.0), 6.51 (d, *J* = 15.0, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.8, 21.6, 28.8, 30.3, 37.2, 42.7, 48.7, 65.2, 64.0, 125.0, 125.7, 126.3, 130.2, 131.4, 132.6, 135.7, 136.5, 137.9.

APPENDIX I [a]

HPLC Columns	HPLC Eluents		Compounds separated, monitoring wavelength (nm)
Silica-based nitrile bonded (Analytical & Semipreparative)	A	75% Hexane, 25% CH_2Cl_2, 0.6% MeOH	**83**/**84**, 260 nm; **81**/**82**, 260 nm; **77** – **80**, 260 nm; **73** – **76**, 280 nm; **64** – **67**, 412 nm; **120** – **123**, 224 nm; **(*3R*)-42**/**(*3S*)-42**, 286 nm.
Silica-based nitrile bonded (Semipreparative)	B	99.9% Hexane, 0.1% Isopropyl alcohol	**83**/**84**, 260 nm
Chiral column	C	95% Hexane, 5% Isopropyl alcohol, 0.75% CH_3CN	**77** – **80**, 260 nm
Chiral column	D	98% CH_3CN, 2% Isopropyl alcohol	**73** – **76**, 280 nm, **64** – **67**, 412 nm
Silica-based nitrile bonded (Semipreparative)	E	75% Hexane, 25% CH_2Cl_2, 0.35% MeOH	**119**, 324 nm, **(*3R*)-42**, 286 nm
C_{18}-Reversed phase (Analytical)	F	85% CH_3CN, 2.5% Hexane, 2.5% CH_2Cl_2, 10% MeOH	**1** – **4**, 446 nm
Chiral column	G	95% Hexane, 5% *tert*-Amyl alcohol	**1**/**2**, 446 nm
Chiral column	H	90% Hexane, 10% 2-Pentanol	**3**/**4**, 446 nm
Chiral column	I	97% Hexane, 3% Isopropyl alcohol, 0.1% CH_3CN	**120** – **123**, 224 nm
Chiral column	J	90% Hexane, 10% Isobutyl alcohol	**(*3R*)-42**/**(*3S*)-42**, 286 nm

APPENDIX I (Continued)

HPLC Columns		HPLC Eluents	Compounds separated, monitoring wavelength (nm)
C_{18}-Reversed phase (Analytical)	K	75% CH_3CN, 20% H_2O, 5% Formic acid	**(*3R*)-16**, **(*3S*)-16**, 286 nm
Chiral column	L	Pump A: 95% Hexane, 5% Isopropyl alcohol Pump B: 85% Hexane, 15% Isopropyl alcohol 90% A, 10% B $\xrightarrow[\text{in 20 mins}]{\text{Time 10}}$ 50% A, 50% B	**5** – **7**, 450 nm
Chiral column	M	97.5% Hexane, 2.5% Isopropyl alcohol	**135/136**, 450 nm
Silica-based nitrile bonded (Semipreparative)	N	75% Hexane, 25% CH_2Cl_2, 0.1% MeOH	**89** – **92**, 360 nm
Silica-based nitrile bonded (Analytical & Semipreparative)	O	90% Hexane, 10% CH_2Cl_2, 0.2% MeOH	**112**, 336 nm; **108** – **111**, 338nm

[a] Details of the HPLC systems, columns, and flow rates are described in the experimental section.

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