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Total Synthesis of (3*R*,3′*R*,6′*R*)-Lutein and Its Stereoisomers

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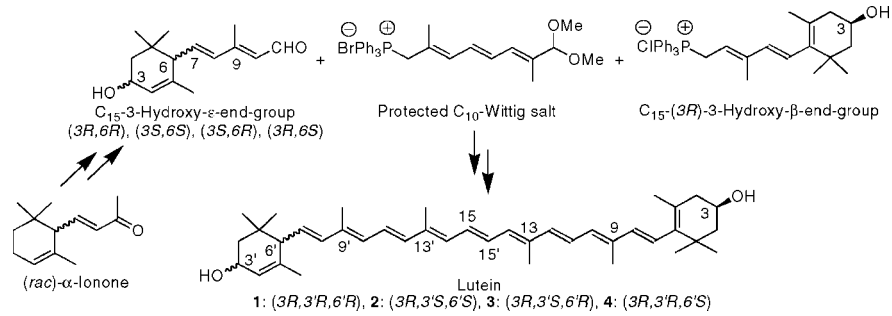

(3*R*,3′*R*,6′*R*)-Lutein (**1**) is a major dietary carotenoid that is abundant in most fruits and vegetables commonly consumed in the U.S. and that accumulates in the human plasma, major organs, and ocular tissues. Numerous epidemiological and experimental studies have shown that **1** has important biological activities and may play an important role in the prevention of age-related macular degeneration (AMD). While the total synthesis of **1** has been previously reported in a poor overall yield, the total synthesis of the other seven stereoisomers of lutein has not yet been accomplished. We have developed a relatively straightforward methodology for the total synthesis of **1** and three of its stereoisomers, (3*R*,3′*S*,6′*S*)-lutein (**2**), (3*R*,3′*S*,6′*R*)-lutein or 3′-epilutein (**3**), and (3*R*,3′*R*,6′*S*)-lutein (**4**) by $C_{15} + C_{10} + C_{15}$ Wittig coupling reactions. Employing this methodology, the other four stereoisomers of lutein that are enantiomeric to the aforementioned lutein isomers can be similarly prepared. One of the important features of this strategy is its application to the total synthesis of ^{13}C-labeled luteins and their metabolites with appropriate stereochemistry for metabolic studies in animals and humans. This synthesis also provides access to the C_{15}-precursors of optically active carotenoids with a 3-hydroxy-ε end group that are otherwise difficult to synthesize.

Introduction

(3*R*,3′*R*,6′*R*)-Lutein (**1**) and (3*R*,3′*R*)-zeaxanthin are two dietary carotenoids that are present in most fruits and vegetables commonly consumed in the U.S.[1] These carotenoids accumulate in human plasma and breast milk,[2] major organs, and ocular tissues[3] and have been implicated in the prevention of age-related macular degeneration (AMD).[4] While (3*R*,3′*R*)-zeaxanthin has been commercially available by total synthesis for more than two decades, the industrial production of **1** by chemical synthesis has not yet materialized.[5] Consequently, this carotenoid is commercially produced from saponified extracts of marigold flowers (*Tagetes erecta*, variety orangeade).[6] The major difficulty with the total synthesis of **1** is due to the presence of three stereogenic centers at the C3, C3′, and C6′ positions in this carotenoid that can result in eight possible stereoisomers (Figure 1).

(1) (a) Khachik, F.; Beecher, G. R.; Goli, M. B.; Lusby, W. R. *Pure Appl. Chem.* **1991**, *63*, 71–80. (b) Humphries, J. M.; Khachik, F. *J. Agric. Food Chem.* **2003**, *51*, 1322–1327. (c) Khachik, F. *Pure Appl. Chem.* **2006**, *78*, 1551–1557.

(2) (a) Khachik, F.; Beecher, G. R.; Goli, M. B.; Lusby, W. R.; Smith, J. C. *Anal. Chem.* **1992**, *64*, 2111–2122. (b) Khachik, F.; Spangler, C. J.; Smith, J. C., Jr.; Canfield, L. M.; Pfander, H.; Steck, A. *Anal. Chem.* **1997**, *69*, 1873–1881.

(3) (a) Bone, R. A.; Landrum, J. T.; Hime, G. W.; Cains, A. *Invest. Ophthalmol. Visual Sci.* **1993**, *34*, 2033–2040. (b) Khachik, F.; Bernstein, P.; Garland, D. L. *Invest. Ophthalmol. Visual Sci.* **1997**, *38*, 1802–1811. (c) Bernstein, P. S.; Khachik, F.; Carvalho, L. S.; Muir, G. J.; Zhao, D. Y.; Katz, N. B. *Exp. Eye Res.* **2001**, *72* (3), 215–223.

(4) (a) Schalch, W.; Dayhaw-Barker, P.; Barker, F. M. *The Carotenoids of Human Macula (Nutritional and Environmental Influences on the Eye)*; Taylor, A., Ed.; CRC Press: Boca Raton, FL, 1999; pp 215−250. (b) Snodderly, D. M.; Auran, J. D.; Delori, F. C. *Invest. Ophthalmol. Visual Sci.* **1984**, *25*, 674–685.

(5) (a) Widmer, E.; Soukup, M.; Zell, R.; Broger, E.; Wagner, H. P.; Imfeld, M. *Helv. Chim. Acta* **1990**, *73*, 861–867. (b) Soukup, M.; Widmer, E.; Lukác, T. *Helv. Chim. Acta* **1990**, *73*, 868–873.

(6) (a) Khachik, F. Process for isolation, purification, and recrystallization of lutein from saponified marigolds oleoresin and uses thereof. U.S. Patent, 5,382,714, 1995.

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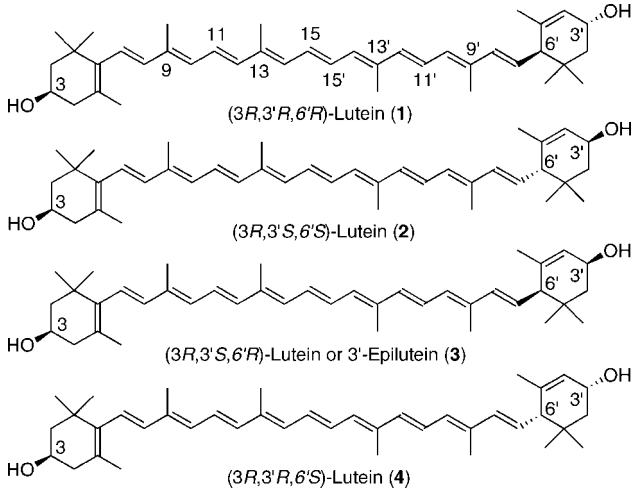


FIGURE 1. Chemical structures of four stereoisomers of lutein.

The other four stereoisomers of lutein (structures not shown) are those in which the configuration at the C3 position is *S* while the stereochemistry at C3′ and C6′ remains the same as in luteins **1**−**4**. Among these stereoisomers, only **1** is of dietary origin, and **3**, a presumed metabolite of **1**, has been identified in human plasma and ocular tissues.[2,3] However, 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 lutein stereoisomers in humans remain unexplored due to the lack of availability of these carotenoids. In 1980, Mayer and Rüttimann reported the only total synthesis of **1** in an overall yield of 1%, and since then no attempt has been made to synthesize this important carotenoid and its stereoisomers. However, it should be noted that 3′-epilutein (**3**) has been prepared by partial synthesis from **1**.[8]

In this paper, we report the total synthesis of luteins **1**−**4** by employing a divergent synthetic strategy that can be extended to the synthesis of the other four stereoisomers of these carotenoids. Our methodology can be applied to the synthesis of ^{13}C-labeled luteins for metabolic studies and provides access to the precursors of optically active carotenoids with a 3-hydroxy-ε end group that are otherwise difficult to prepare.

Nomenclature

The carotenoid numbering system has been used for compounds **15**−**24** to allow comparison of their stereochemical transformations to compounds **1**−**10**. The correct systematic names of these carotenoid precursors followed by their common names are shown in brackets as follows: **15**−**18**, (2*E*,4*E*)-3-methyl-5-(2,6,6-trimethyl-4-hydroxy-2-cyclohexen-1-yl)penta-2,4-dienal [(7*E*,9*E*)-3-hydroxy-α-ionylideneacetaldehyde]; **19**−**22**, (2*E*,4*E*)-3-methyl-5-(2,6,6-trimethyl-4-hydroxy-2-cyclohexen-1-yl)penta-2,4-dienenitrile [(7*E*,9*E*)-3-hydroxy-α-ionylideneacetonitrile]; **23a**: (2*E*,4*E*)-, **23b**: (2*Z*,4*E*)-3-methyl-5-(2,6,6-trimethyl-4-oxo-2-cyclohexen-1-yl)penta-2,4-dienenitrile, [**23a**: (7*E*,9*E*)-, **23b**: (7*E*,9*Z*)-3-keto-α-ionylideneacetonitrile]; **24a**: (2*E*,4*E*)-, **24b**: (2*Z*,4*E*)-3-methyl-5-(2,6,6-trimethyl-2-cyclohexen-1-yl)penta-2,4-dienenitrile, [**24a**: (7*E*,9*E*)-, **24b**: (7*E*,9*Z*)-α-ionylideneacetonitrile].

Retrosynthetic Analysis

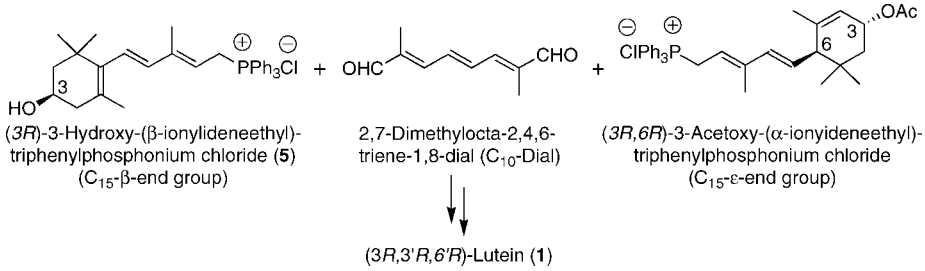
Mayer and Rüttimann employed a $C_{15} + C_{10} + C_{15}$ Wittig coupling reaction for the synthesis of **1** according to Scheme 1.[7] Despite the difficulties encountered in the synthesis of unsymmetrical C_{40}-carotenoids, the $C_{15} + C_{10} + C_{15}$ building block strategy is, in most cases, the method of choice. This is because the formation of the double bonds at the 11 and 11′ positions due to steric interaction yields predominantly the (all-*E*)-isomer of carotenoids.[9]

The (3*R*,6*R*)-3-acetoxy-(α-ionylideneethyl)triphenylphosphonium chloride that was used in the final step of the reported synthesis of **1** was prepared in poor yield and involved numerous steps. In addition, according to Mayer and Rüttimann, the final olefination reaction with this Wittig salt only gave 25% yield of **1**. Therefore, in our synthetic strategy, we employed slightly different C_{15} and C_{10} building blocks to arrive at luteins **1**−**4** (Scheme 2). We anticipated that the final step of our synthesis could be best accomplished by elongation of the optically pure C_{25}-hydroxyaldehydes **6**−**9** with the Wittig salt **5** that could be readily prepared according to the known processes.[5] We rationalized that the optically pure C_{25}-hydroxyaldehydes **6**−**9** could be prepared from deprotection of their corresponding dimethylacetals **10**−**13** under mild acidic conditions without epimerization of their allylic hydroxyl groups at C3. These acetals could in turn be prepared from the reaction of protected Wittig salt **14** with the optically pure C_{15}-hydroxyaldehydes **15**−**18** with the required stereochemistry at C3 and C6. The protected Wittig salt **14** was readily accessible according to published methods.[10]

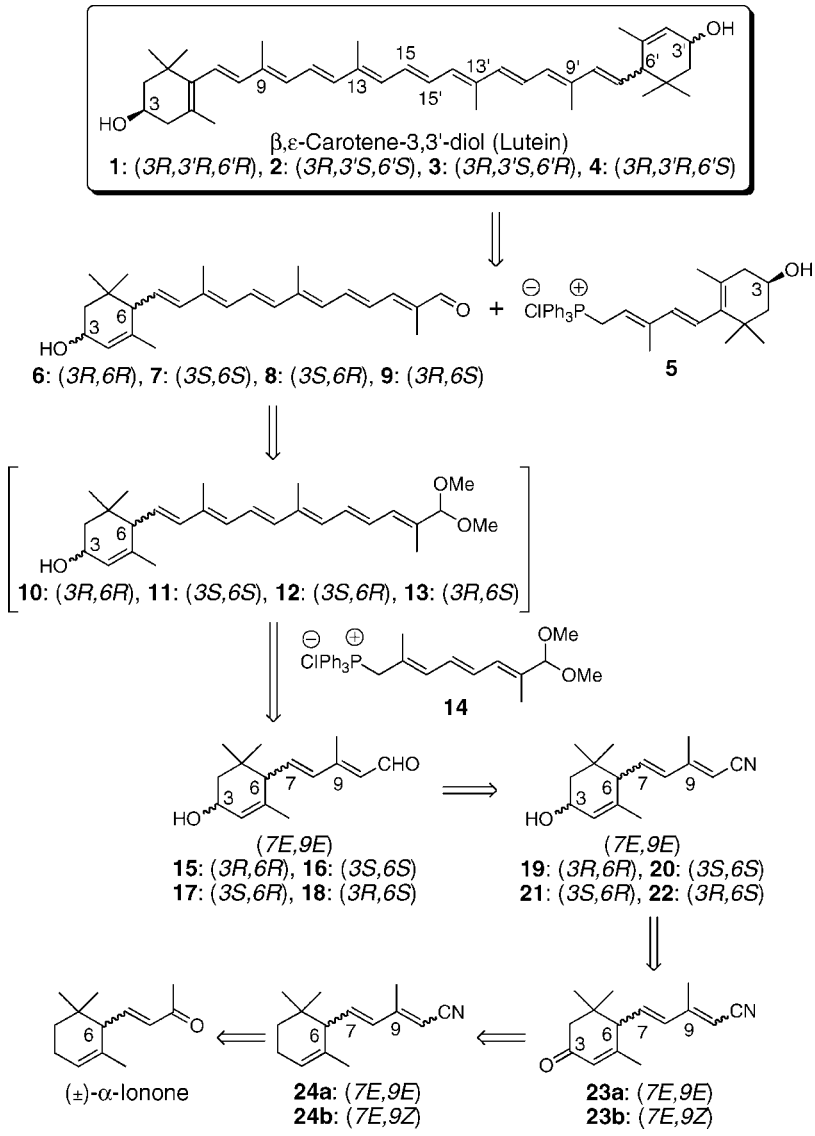
The application of this Wittig salt **14** in the synthesis of unsymmetrical carotenoids with a variety of sensitive functional groups has been well documented in the literature.[10a,11] However, this building block has not been employed in the synthesis of lutein or its precursors. The C_{15}-hydroxynitriles **19**−**22** with the appropriate stereochemistry at C3 and C6 could serve as the precursors of C_{15}-hydroxyaldehydes **15**−**18**. 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. (7*E*,9*E*)-3-Keto-α-ionylideneacetonitrile (**23a**) and its (7*E*,9*Z*)-isomer (**23b**), prepared from nitriles **24a** and **24b**, could be transformed into C_{15}-hydroxynitriles **19**−**22**. However, the (7*E*,9*E*)-isomer (**23a**) would be preferable in order to avoid the difficulties associated with the separation of optically active *E*/*Z*-isomers throughout our entire synthetic strategy. The commercially available and inexpensive (±)-α-ionone was selected as the starting material for the synthesis of nitriles **24a**/**24b**. Ketonitriles **23a** and **23b** have been previously synthesized as a mixture of *E*/*Z*-isomers from (±)-α-ionone by Horner−Wadsworth−Emmons (HWE) olefination.[12] Therefore, we had

(7) Mayer, H.; Rüttimann, A. *Helv. Chim. Acta* **1980**, *63* (6), 1451–1455.
(8) Khachik, F. *J. Nat. Prod.* **2003**, *66*, 67–72.
(9) Soukup, M.; Spurr, P.; Widmer, E. In *Carotenoids, Synthesis*; Britton, G., Liaaen-Jensen, S., Pfander, H., Eds.; Birkhäuser: Basel, 1995; Vol. 2; pp 7−14.
(10) (a) Bernhard, K.; Kienzle, F.; Mayer, H.; Müller, R. K. *Helv. Chim. Acta* **1980**, *63*, 1473–1490. (b) Haugen, J. A. *Acta Chim. Scand.* **1994**, *48*, 657–664.
(11) (a) Haag, A.; Eugster, C. H. *Helv. Chim. Acta* **1985**, *68*, 1897–1906. (b) Yamano, Y.; Sakai, Y.; Yamashita, S.; Ito, M. *Heterocycles* **2000**, *52*, 141–146. (c) Tode, C.; Yamano, Y.; Ito, M. *J. Chem. Soc., Perkin Trans. 1* **2002**, 1581–1587.
(12) Imai, H.; Hirano, T.; Terakita, A.; Shichida, Y.; Muthyala, R. S.; Chen, R. L.; Colmenares, L. U.; Liu, R. S. H. *Photochem. Photobiol.* **1999**, *70*, 111–115.

SCHEME 1



SCHEME 2

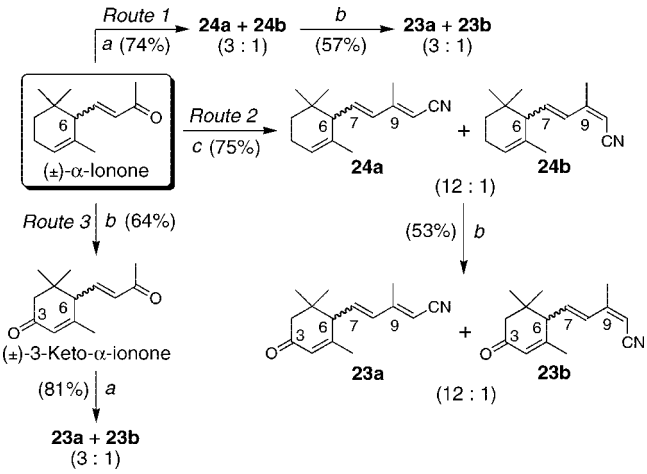


to develop a methodology that could provide **24a** as a single isomer and transform this nitrile into **23a** without stereoisomerization. Other challenges with our synthetic approach were separation of C_{15}-hydroxyaldehydes **15**−**18** or their precursors, C_{15}-hydroxyanitriles **19**−**22**, in high optical purity and maintaining their stereochemical integrity throughout the synthesis of luteins **1**−**4**.

Results and Discussion

One of the key starting materials in the retrosynthetic pathways shown in Scheme 2 is (±)-3-keto-α-ionylideneacetonitrile which had to be preferentially synthesized as the (7*E*,9*E*)-isomer (**23a**) at the expense of its (7*E*,9*Z*)-isomer (**23b**). This is because when (±)-ketonitrile **23a** is reduced in the following step, a new stereogenic center at C3 is generated that results in the formation of four stereoisomers, namely, *rac*-hydroxynitriles **19**−**22**. Consequently, the reduction of a mixture of ketonitriles **23a** and **23b** could afford as many as eight stereoisomeric hydroxynitriles that would be difficult to separate in high optically purity. Therefore, our initial goal was to explore the possible routes by which (±)-α-ionone could be transformed into ketonitrile **23a**. Three synthetic routes were employed for

SCHEME 3[a]



[a] Reagents and conditions: (a) $(EtO)_2P(O)CH_2CN$ or $(^iPrO)_2P(O)CH_2CN$/NaH, TBME; (b) ^{t}BuOOH (TBHP), bleach (5.25% NaOCl), CH_3CN, −5 to 0 °C; (c) $CH_2(CN)CO_2H$, cyclohexylamine, 80−85 °C, 3.5 h.

transformation of (±)-α-ionone to ketonitrile **23a** (Scheme 3). According to the first route, the HWE reaction of (±)-α-ionone with diethyl cyanomethylphosphonate in TBME using NaH as base gave nitriles **24a**:**24b** = 3:1 in 74% isolated yield after distillation. The use of diisopropyl cyanomethylphosphonate has been shown to increase the *E*/*Z* ratio in HWE reaction with *β*-ionone, but in our case this phosphonate only marginally improved the *E*/*Z* ratio.[13]

A mixture of nitriles **24a** and **24b** was oxidized with ^{t}BuOOH (TBHP, 70% in water), bleach, and catalytic amounts of K_2CO_3 in CH_3CN at −5 to 0 °C to yield **23a**:**23b** = 3:1. 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 **23a** as white crystals free from **23b** in 37% isolated yield. This water-based oxidation system, using household laundry bleach and aqueous TBHP, has been shown to convert steroidal olefins to α,*β*-enones by an economical and environmentally friendly methodology.[14] Ketonitriles **23a** and **23b** were also prepared in 53% yield by palladium(II)-mediated oxidation of nitriles **24a** and **24b** with TBHP in CH_2Cl_2 at 0 °C, a methodology similar to one that has been employed for allylic oxidation of olefins.[15] However, to date there is no literature report on the direct oxidation of nitriles **24a** and **24b** to ketonitriles **23a** and **23b**. These reactions clearly revealed that oxidation of a mixture of **24a** and **24b** to **23a** and **23b** is not accompanied by *E*/*Z*-isomerization and the isomeric ratio of these nitriles remained unchanged.

In search of an alternative process that could provide **24a** as a single isomer, we turned our attention to Knoevenagel condensation. 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.[16] Unfortunately, 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.[17] 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 **24a**: **24b** = 12: 1 as a colorless oil after distillation (Route 2, Scheme 3). In the following step, the mixture of these nitriles in CH_3CN was oxidized with TBHP and household bleach as described earlier to yield ketonitriles **23a** (92%) and **23b** (8%) in 53% isolated yield. After low temperature (−15 °C) crystallization from ethanol, (*7E,9E*)-ketonitrile **23a** was obtained as white crystals in 37% isolated yield and contained no measurable amounts of **23b**.

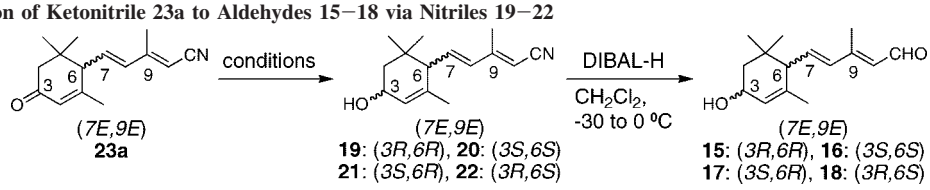
We also investigated the HWE reaction of (±)-3-keto-α-ionone with diethyl cyanomethylphosphonate in THF according to the method of Imai et al.[12] and obtained **23a**: **23b** = 3: 1 in 81% yield (Route 3, Scheme 3). After purification by chromatography and crystallization from ethanol, **23a** was obtained as white crystals in 40% isolated yield. It should be noted that Imai et al. prepared **23a** and **23b** as a mixture and the isomeric ratio of these ketonitriles were not reported. (±)-3-Keto-α-ionone was first prepared in 1952 from allylic oxidation of (±)-α-ionone in 14% isolated yield by Prelog and Osgan.[18] Widmer et al. employed $Ac_2Co.4H_2O/NH_4Br/O_2$ to improve the yield of this reaction to 31%.[19] 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 (±)-3-keto-α-ionone in 67% isolated yield.[20] Employing bleach oxidation, we were able to prepare crystalline (±)-3-keto-α-ionone from (±)-α-ionone in 64% isolated yield. The palladium(II)-mediated oxidation of (±)-α-ionone with TBHP in CH_2Cl_2 also afforded this ketone in 53% isolated yield.

While the overall yield of the three routes discussed above were comparable, route 2 proved to be easier to scale up and due to the high *E*-stereoselectivity of the Knoevenagel condensation, **23a** could be crystallized from the isomeric mixture more expeditiously.

Reduction of (7*E*,9*E*)-3-Keto-α-ionylideneacetonitrile (23a) to (7*E*,9*E*)-3-Hydroxy-α-ionylideneacetaldehydes 15−18 via (7*E*,9*E*)-3-Hydroxy-α-ionylideneacetonitriles 19−22. (7*E*,9*E*)-Ketonitrile **23a** was reduced to four stereoisomeric hydroxynitriles **19**−**22** with a number of reagents in high yields (see Table 1S, Supporting Information). Because (3*R*,6*R*)-hydroxynitrile **19** with a *trans* relationship between the OH at C3 and C6-dienenitrile side chain is the precursor of the naturally occurring (3*R*,3′*R*,6′*R*)-lutein (**1**), it was desirable to increase the composition of the (3,6-*trans*)-hydroxynitriles **19** and **20** relative to the (3,6-*cis*)-hydroxynitriles **21** and **22**. Among the reducing agents employed, potassium tri-*sec*-butylborohydride (K-Selectride)[21] produced the greatest amounts of the (3,6-*trans*)-hydroxynitriles relative to the (3,6-*cis*)-hydroxynitriles [(**19**+**20**):(**21**+**22**) = 6:1]

(13) Dugger, R. W.; Heathcock, C. H. *Synth. Commun.* **1980**, *10*, 509–515.
(14) Marwah, P.; Marwah, A.; Lardy, H. A. *Green Chem.* **2004**, *6*, 570–577.
(15) Yu, J. Q.; Corey, E. J. *Org. Lett.* **2002**, *4*, 2727–2730.
(16) Young, W. G.; Andrews, L. J.; Cristol, S. J. *J. Am. Chem. Soc.* **1944**, *66*, 520–524.
(17) Andriamialisoa, Z.; Valla, A.; Zennache, S.; Giraud, M.; Potier, P. *Tetrahedron Lett.* **1993**, *34*, 8091–8092.
(18) Prelog, V.; Osgan, M. *Helv. Chim. Acta* **1952**, *35*, 986–992.
(19) Widmer, E.; Soukup, M.; Zell, R.; Broger, E.; Lohri, B.; Marbet, R.; Lukác, T. *Helv. Chim. Acta* **1982**, *65*, 944–957.
(20) Yang, M.; Peng, Q. R.; Lan, J. B.; Song, G. F.; Xie, R. G. *Synlett* **2006**, *16*, 2617–2620.
(21) (a) Dauben, W. G.; Ashmore, J. W. *Tetrahedron Lett.* **1978**, 4487–4490. (b) Bell, R. A.; Turner, J. V. *Tetrahedron Lett.* **1981**, *22*, 4871–4872. (c) Göndös, G.; Orr, J. C. *J. Chem. Soc., Chem. Commun.* **1982**, 1239–1240. (d) Tal, D. M.; Frisch, G. D.; Elliott, W. H. *Tetrahedron* **1984**, *40*, 851–854.

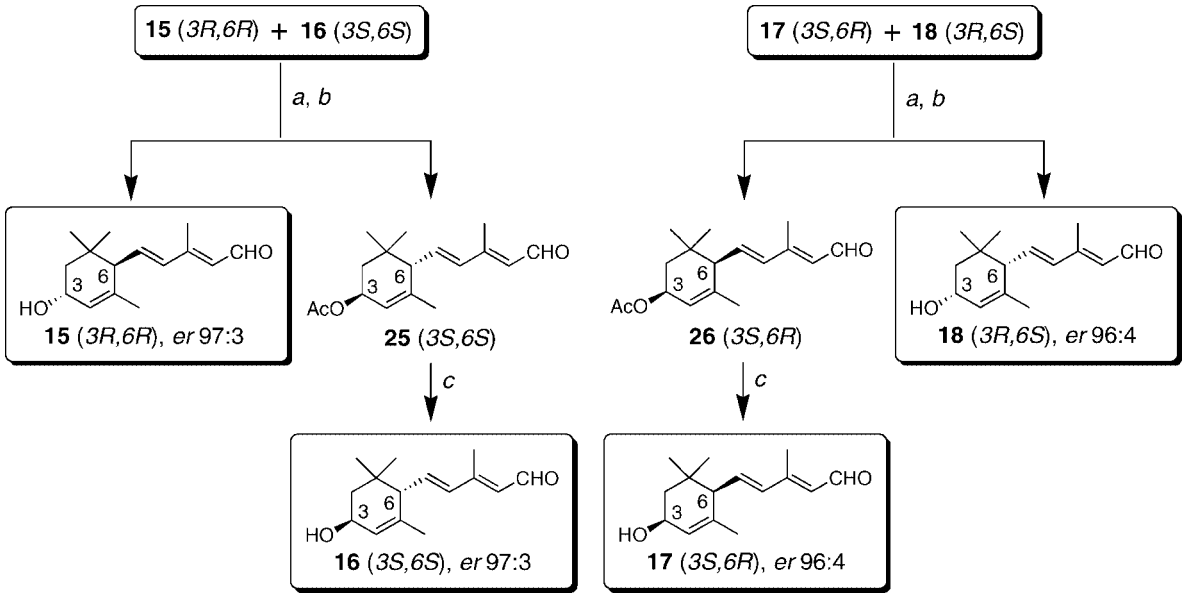
TABLE 1. Reduction of Ketonitrile 23a to Aldehydes 15−18 via Nitriles 19−22



entry	conditions	yield,[a] (**19**+**20**):(**21**+**22**)[b]	yield,[a] (**15**+**16**):(**17**+**18**)[b]
1	KB[CHMeC$_2$H$_5$]$_3$H, TBME, −30 °C, 4 h	94%, 6:1	70%, 6:1 83%, 6:1 (one-pot process)
2	(*R*)-2-methyl-CBS-oxaza-borolidine/BH_3, TBME, 0 °C, 1.5 h	97%, 1:6	80%, 1:6

[a] Isolated yield after chromatography. [b] Ratios were determined by HPLC on a silica-based nitrile bonded column and a chiral column (see Supporting Information).

SCHEME 4[a]



[a] Reagents and conditions: (a) lipase AK (*Pseudomonas fluorescens*), vinyl acetate, pentane (reflux), 48 h, 43% conversion; (b) chromatography (*n*-Si, hexane/EtOAc, 98:2 to 85:15); (c) KOH/MeOH (10%, w:v), 0 °C, 2 h, 97%.

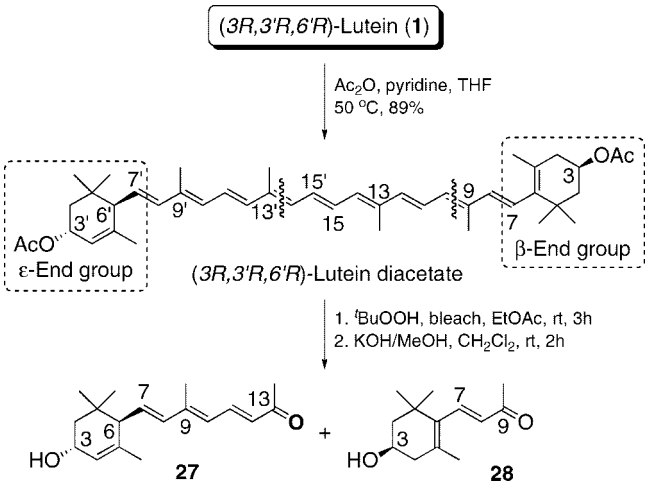
(Table 1). When Corey's enantiomerically pure (*R*)-2-methyl-CBS-oxazaborolidine[22] was used as the reducing agent, this diastereoselectivity was reversed and the (3,6-*cis*)-hydroxynitriles were the major products [(**19**+**20**):(**21**+**22**) = 1:6]. Hydroxynitriles **19**−**22** were subsequently reduced to their corresponding hydroxyaldehydes **15**−**18** (Table 1).

Interestingly, the reduction of **23a** with (*S*)-2-methyl-CBS-oxazaborolidine under the same conditions (Table 1) gave (**15**+**16**):(**17**+**18**) = 1:3 and stereoselectivity was not reversed. The separation of the (3,6-*trans*)-hydroxynitriles (**19**+**20**) from the (3,6-*cis*)-hydroxynitriles (**21**+**22**) by column chromatography proved to be challenging and could only be accomplished with repeated chromatography. Therefore, the hydroxynitriles **19**−**22** were first transformed into a racemic mixture of hydroxyaldehydes **15**−**18** by DIBAL-H and the mixture was then subjected to column chromatography. Unlike hydroxynitriles, the (3,6-*trans*)-hydroxyaldehydes (**15**+**16**) were readily separated from the (3,6-*cis*)-hydroxyaldehydes (**17**+**18**) by chromatography. In an alternative one-pot reaction, ketonitrile **23a** was reduced to hydroxynitriles **19**−**22** with K-Selectride followed by the reduction with DIBAL-H to afford hydroxyaldehydes **15**−**18** in one convenient step (Table 1).

Separation of Hydroxyaldehydes 15−18 in High Optical Purity by Enzyme-Mediated Acylation. We have previously demonstrated that when a diastereomeric mixture of (3*R*,3′*S*,6′*R*)-lutein (**1**) and (3*R*,3′*S*,6′*R*)-lutein (**3**) is subjected to enzyme-mediated acylation with lipase AK (*Pseudomonas fluorescens*), **1** remains unreacted while the allylic hydroxyl group in **3** is readily acylated. This allowed the separation of **3** from **1** in diastereomeric ratio (dr) of 95:5.[8] Because the allylic hydroxyl group in **3** with *3S* configuration was shown to be highly reactive toward enzymatic acylation while the *3R*-hydroxyl group in **1** was unreactive, this approach was used to resolve the racemic mixture of hydroxyaldehydes **15**−**18**. When a racemic mixture of **15** and **16** was subjected to enzyme-mediated acylation with lipase AK in the presence of vinyl acetate in refluxing pentane, **16** was acylated to acetoxyaldehyde **25** within 48 h, while **15** remained unchanged and was obtained in high optical purity (er 97:3) (Scheme 4). Acetoxyaldehydes **25** was then readily separated from **15** by column chromatography and was subjected to saponification with KOH/MeOH at 0 °C to afford **16** (er 97:3). Similarly, the resolution of a racemic mixture of **17** and **18** was accomplished with enzymatic acylation under the same conditions. Hydroxyaldehyde **17** underwent acylation to acetoxyaldehyde **26**, while **18** was found to be unreactive. After column chromatography and saponification of **26**, hydroxyaldehydes **17** (er 96:4) and **18** (er 96:4) were obtained in high optical purity.

(22) (a) Corey, E. J.; Bakshi, R. K.; Shibata, S. *J. Am. Chem. Soc.* **1987**, *109*, 5551–5553. (b) Corey, E. J.; Bakshi, R. K.; Shibata, S.; Chen, C. P.; Singh, V. K. *J. Am. Chem. Soc.* **1987**, *109*, 7925–7926.

SCHEME 5



Determination of the Absolute Configuration of Hydroxyaldehydes 15−18. Hydroxyldehydes **15**−**18** 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 (3*R*,3′*R*,6′*R*)-lutein (**1**) and (3*R*,3′*S*,6′*R*)-lutein (**3**).[23] It has been well documented that when H-6 and the hydroxyl group at C-3 in lutein 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. The chemical shift of H-6 proton in (3,6-*trans*)-aldehydes **15** and **16** in which this proton is in a *cis*-geometry with the hydroxyl group at C-3 appeared at $\delta = 2.50$ ppm while this chemical shift in (3,6-*cis*)-aldehydes **17** and **18** (H-6 and OH in *trans*-geometry) moved upfield to $\delta = 2.25$ ppm. Therefore, the relative stereochemistry of hydroxyldehydes **15**−**18** 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 (3*R*,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 5).[24]

Prior to oxidative cleavage of **1**, the hydroxyl groups were protected by acylation with Ac_2O-pyridine and the resulting (3*R*,3′*R*,6′*R*)-lutein diacetate was then oxidized with TBHP/bleach. After saponification and column chromatography, HPLC analysis of the product showed the presence of unreacted **1** (80%) and a number of oxidation products. Among these, (3*R*,6*R*)-3-hydroxy-13-apo-ε-caroten-13-one (**27**) with an ε end group and (3*R*)-3-hydroxy-β-ionone (**28**) with a β end group were the only major stable products (**27**:**28** = 3:1). These were separated by semipreparative HPLC and were fully characterized from their NMR, MS, UV−vis, and CD spectra. The CD spectra of (3,6-*trans*)-aldehydes **15** (3*R*,6*R*) [281 nm (+18 mdeg), 242 nm (−1.3 mdeg)] and **16** (3*S*,6*S*) [281 nm (−13 mdeg), 242 nm (+1.0 mdeg)] in ether−hexane−methanol (10:3:1) with strong opposite Cotton effects clearly indicated that these aldehydes were enantiomeric. The absolute configuration of **15** was assigned as (3*R*,6*R*) by comparison of its CD spectrum with that of hydroxyketone **27** (3*R*,6*R*) [320 nm (+10.6 mdeg)] that similar to this aldehyde showed a positive Cotton effect.

To establish the absolute configuration of **17** and **18**, aldehydes **15** and **17** were separately epimerized in dilute aqueous HCl similarly to our previously reported epimerization of (3*R*,3′*R*,6′*R*)-lutein (**1**) to 3′-epilutein (**3**).[8] Under identical reaction conditions, the HPLC analysis of the crude product revealed that hydroxyaldehydes **15** (32%) and **17** (68%) had reached an equilibrium that favored the (3,6-*cis*)-aldehyde **17** (Scheme 6).

The products of these reactions were then separated by semipreparative HPLC, and their absolute configurations were determined by NMR and CD. The H-6 chemical shift of the epimer of **15** appeared at $\delta = 2.25$ ppm, indicating a *trans*-geometry between H-6 and OH, and on this basis this epimer was identified as **17** (3*S*,6*R*). The CD spectrum of the epimer of **15** was also identical to that of the synthetic sample of **17** that was prepared in high optical purity by enzymatic acylation of the racemic mixture of **17** and **18** (Scheme 4). Similarly, the epimer of **17** was shown by NMR and CD to be identical to **15**. Because the CD spectra of **17** (3*S*,6*R*) and **18** indicated that these aldehydes were enantiomeric, the absolute configuration of **18** was assigned as *3R,6S*.

Synthesis of Luteins 1−4 via C_{25}-Hydroxy-apocarotenals 6−9. The C_{15}-hydroxyaldehydes **15**−**18** prepared in high optical purities were each elongated to their corresponding protected C_{25}-aldehydes **10**−**13** by olefination with the protected Wittig salt **14** in the presence of NaOMe/MeOH at rt (Scheme 7). After solvent evaporation and without isolation of the products, acetals **10**−**13** 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 **6**−**9** as a mixture of all-*E* and 11*Z*. Fortunately, the (11*Z*)-isomers of carotenoids and apocarotenoids are sterically hindered and are readily converted to their (all-*E*)-isomers.[25] Thus, prior to purification, the crude mixture of (all-*E*)- and (11*Z*)-isomers of each of these aldehydes were catalytically isomerized to their corresponding (all-*E*)-isomers in the presence of $Pd(OAc)_2$ in refluxing EtOAc within 2 h. In the following step, the individual aldehydes were purified by column chromatography to afford **6**−**9** in isolated yields ranging from 53−85%. Aldehydes **6** and **7** were each obtained in an er of 97:3, and similarly **8** and **9** were each prepared in an er of 96:4. It should be noted that under the acidic conditions employed for the deprotection of acetals **10**−**13**, the hydroxyl group at C3 did not undergo epimerization and the optical purities of the resulting C_{25}-aldehydes **6**−**9** were not compromised. This was confirmed by chiral HPLC (Supporting Information) of the individually synthesized (all-*E*)-C_{25}-aldehydes.

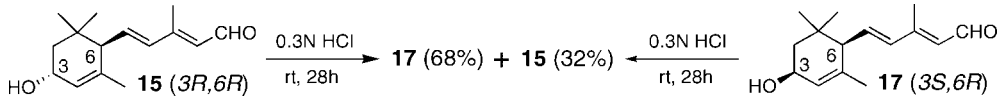
Although the (11*Z*)-isomers of C_{25}-hydroxyaldehydes **6**−**9** were readily isomerized into their corresponding (all-*E*)-isomers, this step was shown to be unnecessary and could be postponed until after preparation of luteins **1**−**4**.

(23) (a) Buchecker, R.; Eugster, C. H. *Helv. Chim. Acta* **1979**, *62*, 2817–24. (b) Englert, G. In *Carotenoid Chemistry and Biochemistry*; Britton, G., Goodwin, T. W., Eds.; Pergammon: Oxford, 1982; pp 107−134. (c) Englert, G. In *Carotenoids*; Britton, G., Liaaen-Jensen, S., Pfander, H., Eds.; Birkhäuser: Basel, 1995; Vol. 1B, pp 147−260. (d) Khachik, F.; Englert, G.; Daitch, C. E.; Beecher, G. R.; Lusby, W. R.; Tonucci, L. H. *J. Chromatogr. Biomed. Appl.* **1992**, *582*, 153–166.

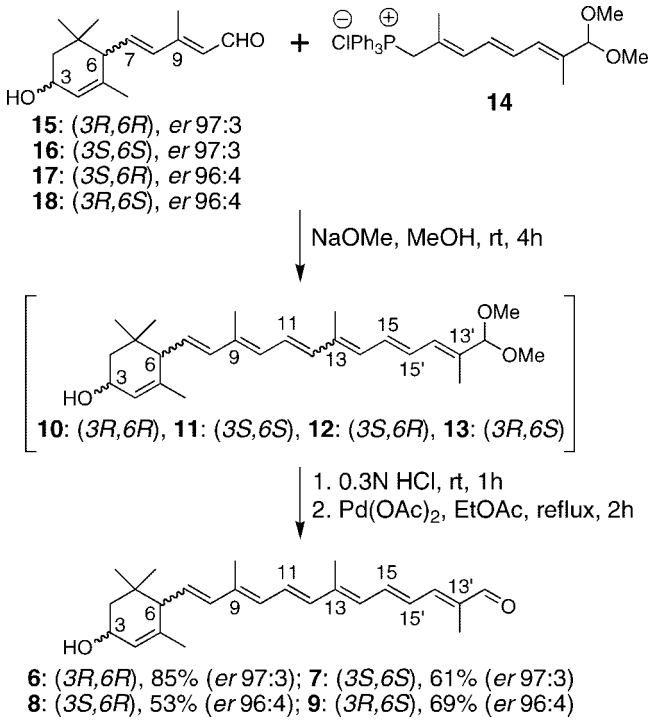
(24) (a) Buchecker, R.; Hamm, P.; Eugster, C. H. *Chimia* **1971**, *25*, 192–194. (b) Buchecker, R.; Hamm, P.; Eugster, C. H. *Chimia* **1972**, *26*, 134–136. (c) Buchecker, R.; Hamm, P.; Eugster, C. H. *Helv. Chim. Acta* **1974**, *57*, 631–656.

(25) Soukup, M.; Spurr, P.; Widmer, E. In *Carotenoids*; Britton, G., Liaaen-Jensen, S., Pfander, H., Eds.; Birkhäuser: Basel, 1995; Vol. 2, pp 7−14.

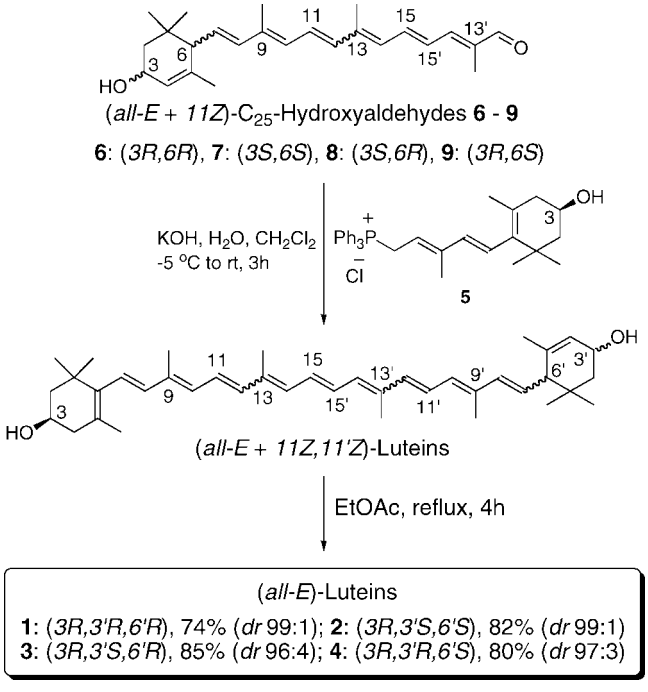
SCHEME 6



SCHEME 7



SCHEME 8



Therefore in a simplified process, each of the (all-*E* + 11*Z*)-C_{25}-hydroxyaldehydes **6**−**9** were allowed to react with the Wittig salt **5**[5] to yield their corresponding luteins **1**−**4** as a mixture of (all-*E*)- and (11*Z*,11′*Z*)-isomers (Scheme 8). The crude mixture of each *E*/*Z*-lutein was then thermally isomerized in a refluxing solution of EtOAc to afford its corresponding (all-*E*)-isomer. After chromatography and crystallization, luteins **1** (74%, dr 99:1), **2** (82%, dr 99:1), **3** (85%, dr 96:4), and **4** (80%, dr 97:3) were prepared in high optical purities. The ^{1}H and ^{13}C NMR spectra of luteins **1**−**4** were in agreement with the published NMR data of the polyene chain and the end groups of these carotenoids.[7,23] The CD spectrum of **1** was also in agreement with that of naturally occurring (3*R*,3′*R*,6′*R*)-lutein, as well as the CD spectrum of this carotenoid reported by Mayer.[23c]

The overall yield of luteins **1**−**4** according to our synthetic strategy can be determined on the basis of the stereochemistry of the targeted lutein. For example, if the synthesis of (3*R*,3′*R*,6′*R*)-lutein (**1**) and (3*R*,3′*S*,6′*S*)-lutein (**2**) is of particular interest, the one-pot reduction of ketonitrile **23a** with K-Selectride followed by DIBAL-H would be the preferred route. This is because this reduction predominantly provides the (3,6-*trans*)-hydroxyaldehydes **15** and **16** 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 **23a** were determined as follows: **23a** → (**15** + **16**), 71% → **15** (30%) and **16** (31%) → **6** (26%) and **7** (28%) → **1** (18%) and **2** (20%). Because the isolated yield of ketonitrile **23a** 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 **23a** with (*R*)-2-methyl-CBS-oxazaborolidine is preferred because this reagent provides the (3,6-*cis*)-hydroxyaldehydes **17** and **18** as the major products. According to this route, the overall yields of luteins **3** and **4** based on ketonitrile **23a** were 17% and 19%, respectively. However, the calculated yields of **3** (5%) and **4** (6%) based on (±)-α-ionone were significantly lower.

Although **23a** served as the key starting material in our synthesis, the preparation and crystallization of this (7*E*,9*E*)-isomer from (±)-α-ionone in a low yield (28%) contributed to the low overall yield of luteins **1**−**4**.

Conclusion

The same strategy that we have employed for the synthesis of luteins **1**−**4** can also be used to elongate C_{25}-hydroxyaldehydes **6**−**9** with the *S*-enantiomer of Wittig salt **5** to synthesize the other four stereoisomers of luteins; these are (3*S*,3′*S*,6′*S*)-lutein, (3*S*,3′*R*,6′*R*)-lutein, (3*S*,3′*R*,6′*S*)-lutein, and (3*S*,3′*S*,6′*R*)-lutein. Further, the hydroxyaldehydes **15**−**18** provide access to the synthesis of all eight stereoisomers of lutein labeled with carbon-13 for metabolic studies. This is because we have previously demonstrated that 2,7-dimethylocta-2,4,6-triene-1,8-dial, the precursor to Wittig salt **14**, can be labeled with 4−6 carbon-13 and employed in the synthesis of (3*R*,3′*R*)-zeaxanthin by C_{15} + C_{10} + C_{15} Wittig coupling strategy.[26] This will allow

(26) Khachik, F.; Beecher, G. R.; Li, B. W.; Englert, G. *J. Labelled Compd. Radiopharm.* **1995**, *36*, 1157–1172.

(27) (a) Khachik, F.; Beecher, G. R.; Smith, J. C., Jr. *J. Cell. Biochem.* **1995**, *22*, 236–246. (b) Khachik, F.; De Moura, F.; Zhao, D. Y.; Aebischer, C. P.; Bernstein, P. S. *Invest. Ophthalmol. Visual Sci.* **2002**, *43*, 3383–3392. (c) Khachik, F.; London, E.; De Moura, F.; Johnson, M.; Steidl, S.; DeTolla, L.; Shipley, S.; Sanchez, R.; Chen, X. Q.; Flaws, J.; Lutty, G.; McLeod, S.; Fowler, B. *Invest. Ophthalmol. Visual Sci.* **2006**, *47*, 5476–86.

the introduction of 4−6 carbon-13 into the polyene chain of luteins **1**−**4** according to our synthetic methodology. Based on our human and animal supplementation studies with naturally occurring lutein, we have previously demonstrated that a minimum of 4−6 carbon-13 randomly located in the lutein polyene chain would be sufficient for investigating the ocular metabolism of this carotenoid.[27]

The U.S. National Eye Institute is currently conducting a multicenter, randomized clinical trial to assess the effects of oral supplementation of (3*R*,3′*R*,6′*R*)-lutein (**1**) and (3*S*,3′*R*)-zeaxanthin on the progression to advanced age-related macular degeneration (AMD) (http://www.areds2.org). Therefore, the availability of a methodology that can be applied to the synthesis of isotopically labeled lutein and its stereoisomers for metabolic studies in an appropriate animal model is essential. Finally, our synthesis provides a relatively easy access to hydroxyaldehydes **15**−**18** that are key starting materials in the synthesis of naturally occurring carotenoids with 3-hydroxy-ε end groups.

Experimental Section

General Materials and Methods. See Supporting Information. (±)-α-Ionone is commercially available. Compounds **5**[5] and **14**[10a] were prepared according to published procedures. Naturally occurring (3*R*,3′*R*,6′*R*)-lutein (**1**) was isolated from a saponified extracts of marigold flowers and was purified by crystallization.[6a] Circular dichroism (CD) spectra were obtained in a mixture of hexane, ether, and methanol (10:3:1).

α-Ionylideneacetonitrile (24a/24b) from (±)-α-Ionone by WHE Coupling (Route 1). 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 rt for 1 h, the mixture was cooled in an ice bath, and freshly distilled (±)-α-ionone (38.10 g, 0.198 mol) in TBME (20 mL) was added in 45 min at 0−5 °C. The mixture was stirred at rt for 4 h, and the product was quenched with water, extracted with TBME, dried, and concentrated to give 45.4 g of a pale yellow oil. The crude product was purified by fractional distillation to yield a mixture of **24a** and **24b** (bp = 107−110 °C at 10 mm) as a colorless oil (31.60 g, 0.147 mol, 74%); **24a**:**24b** = 3:1 (determined by HPLC). The ^{1}H and ^{13}C NMR peak assignments were made by comparison of the spectra of the 3:1 mixture with those of a mixture of **24a**:**24b** = 12:1 that was prepared by an alternative method (Route 2, described later).

24a: ^{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); HRMS (ESI^+) calcd for $C_{15}H_{21}N$ 216.17468 $[M + H]^+$, found 216.17268.

24b: ^{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); HRMS (ESI^+) calcd for $C_{15}H_{21}N$ 216.17468 $[M + H]^+$, found 216.17423.

α-Ionylideneacetonitrile (24a/24b) from (±)-α-Ionone and Cyanoacetic Acid (Route 2). Freshly distilled (±)-α-ionone (36.00 g, 0.187 mol) in cyclohexylamine (62.0 mL, 53.75 g, 0.542 mol) was treated with cyanoacetic acid (20.25 g, 0.238 mol), 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 quenched with water and extracted with hexane. The combined organic solution was dried and concentrated to give 38.00 g of a pale yellow oil that was purified by fractional distillation to yield a mixture of **24a/24b** = 12/1 (bp = 105−110 °C at 10 mm) as a colorless oil (30.00 g, 0.139 mol, 75%). The NMR data were shown to be identical with those of **24a/24b** characterized earlier.

Bleach Oxidation of (±)-α-Ionylideneacetonitrile to 3-Keto-α-ionylideneacetonitrile (23a/23b) (Route 2). A mixture of (±)-α-ionylideneacetonitrile (27.00 g, 0.125 mol; **24a**:**24b** = 12:1) and K_2CO_3 (1.71 g, 12.37 mmol) in acetonitrile (103.0 mL, 80.95 g, 1.972 mol) was cooled to 0 °C under N_2, and a 70% solution of TBHP in water (124 mL, 111.6 g, 70% ≈ 78.12 g, 0.867 mol) was added dropwise in 30 min. Household bleach containing 5.25% NaOCl (386 g, 20.265 g NaOCl, 0.272 mol) was 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 1 h, the product was extracted with hexane and washed with water, and the combined organic solution was dried and concentrated to give 36.7 g of a yellow oil. The crude product was purified by column chromatography (*n*-silica, hexane/ethyl acetate, from 98:2 to 92:8) to yield a mixture of (7*E*,9*E*)-3-keto-α-ionylideneacetonitrile (**23a**) and (7*E*,9*Z*)-3-keto-α-ionylideneacetonitrile (**23b**) (15.19 g, 66.24 mmol, 53%, **23a**:**23b** = 12:1) as a yellow oil. Crystallization from ethanol at −15 °C gave **23a** (10.50 g, 45.79 mmol, 37%) as a white solid, mp 93−95 °C. ^{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). HRMS (ESI^+) calcd for $C_{15}H_{19}NO$ $[M + H]^+$ 230.15394, found 230.15272. The mother liquor from the above crystallization was subjected to semipreparative HPLC to yield an analytically pure sample of **23b**. ^{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). HRMS (ESI^+) calcd for $C_{15}H_{19}NO$ $[M + H]^+$ 230.15394, found 230.15330.

Reduction of (7*E*,9*E*)-3-Keto-α-ionylideneacetonitrile (23a) to Hydroxynitriles 19−22 with K-Selectride. A solution of **23a** (3.00 g, 13.08 mmol) in TBME (25 mL) at −30 °C under N_2 was treated dropwise with a 1 M solution of K-Selectride in THF (20 mL, 20 mmol) in TBME (10 mL) in 40 min. After 4 h at this temperature, the product was quenched with 15 mL of 3 N NaOH followed by 15 mL of 30% H_2O_2 and stirred at rt for 30 min. The product was extracted with TBME, washed with brine, dried, and concentrated to give a colorless oil that was passed through a short silica gel column (hexane/acetone = 97:3) to afford 3-hydroxy-α-ionylideneacetonitriles **19**−**22** (2.85 g, 12.32 mmol, 94%). The isomeric ratio of hydroxynitriles (**19**+**20**):(**21**+**22**) = 6:1 was established by normal phase HPLC. The mixture was subjected to three additional column chromatography separations (hexane/acetone = 97:3) to separate (3,6-*trans*)-hydroxynitriles **19** + **20** (2.14 g) from (3,6-*cis*)-hydroxynitriles **21** + **22** (0.36 g). Hydroxynitriles **19** + **20** and **21** + **22** were each shown by chiral HPLC to consist of an approximately 1:1 mixture of enantiomers.

(3,6-*trans*,7*E*,9*E*)-3-Hydroxy-α-ionylideneacetonitriles (19 + 20). ^{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). HRMS (ESI^+) calcd for $C_{15}H_{21}NO$ $[M + H]^+$ 232.16959, found 232.16997.

(3,6-*cis*,7*E*,9*E*)-3-Hydroxy-α-ionylideneacetonitriles (21 + 22). ^{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). HRMS (ESI$^+$) calcd for $C_{15}H_{21}NO$ $[M + H]^+$ 232.16959, found 232.17062, and $[(M + H) - H_2O]^+$ 214.15944.

Reduction of (7*E*,9*E*)-3-Hydroxy-α-ionylideneacetonitriles 19−22 to (7*E*,9*E*)-3-Hydroxy-α-ionylideneacetaldehydes 15−18. To a solution of hydroxynitriles **19**−**22** [2.30 g, 9.94 mmol, (**19**+**20**):(**21**+**22**) = 6:1] in CH_2Cl_2 (10 mL) was added a 1 M solution of DIBAL-H in CH_2Cl_2 (33 mL, 33 mmol) at −40 °C under N_2 in 1 h. The reaction mixture was stirred at −30 °C for 1 h, and the mixture was very slowly treated with a homogeneous mixture of 26 g of water absorbed on *n*-silica (0.3 g of water/g of silica) at a rate such that the temperature remained below −10 °C. **Caution**: the addition of silica/water results in rapid elevation of the temperature. The reaction mixture was then allowed to stir at 0 °C for 2 h. Na_2SO_4 (3.0 g) was added, and the solids were filtered off and washed with CH_2Cl_2. The organic solution was washed with water, dried, and concentrated to give a pale yellow oil (2.00 g) that was purified by column chromatography (hexane/ethyl acetate, 95:5 to 80:20) to yield (3,6-*trans*)-hydroxyaldehydes **15** + **16** (1.40 g, 5.97 mmol, 60%) and (3,6-*cis*)-hydroxyaldehydes **17** + **18** (0.23 g, 0.98 mmol, 10%).

(3,6-*trans*,7*E*,9*E*)-3-Hydroxy-α-ionylideneacetaldehyde (15 + 16). ^{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, 136.0, 136.3, 138.3, 153.9, 191.4. UV λ_{max} = 280 nm (hexane). HRMS (ESI$^+$) calcd for $C_{15}H_{22}O_2$ $[M + H]^+$ 235.16926, found 235.17037, and $[(M + H) - H_2O]^+$ 217.15933.

(3,6-*cis*,7*E*,9*E*)-3-Hydroxy-α-ionylideneacetaldehyde (17 + 18). ^{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). HRMS (ESI$^+$) calcd for $C_{15}H_{22}O_2$ $[M + H]^+$ 235.16926, found 235.16888, and $[(M + H) - H_2O]^+$ 217.15927.

Enzyme-Mediated Acylation of (3,6-*trans*,7*E*,9*E*)-3-Hydroxy-α-ionylideneacetaldehydes 15 + 16 with Lipase AK (*Pseudomonas fluorescens*). To a solution of (3,6-*trans*)-hydroxyaldehydes **15** + **16** (2.40 g, 10.24 mmol) in 20 mL of pentane were 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 acylation was monitored by chiral HPLC. After 48 h, the product was filtered through Celite, and the filtrate was concentrated 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. The first fraction was identified as (3*S*,6*S*)-3-acetoxy-α-ionylideneacetaldehyde (**25**) (1.22 g, 4.41 mmol; 43%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.90 (s, 3H), 1.02 (s, 3H), 1.49 (dd, J = 14.0, 5.3, 1H), 1.65 (s, 3H), 1.84 (dd, J = 14.0, 6.0, 1H), 2.05 (s, 3H), 2.26 (s, 3H), 2.50 (d, J = 9.8, 1H), 5.33 (m, 1H), 5.55 (s, 1H), 6.01 (dd, J = 15.6, 9.8, 1H), 6.22 (d, J = 15.6, 1H), 10.12 (d, J = 8.0, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.2, 21.4, 22.8, 25.4, 28.8, 33.5, 39.3, 54.9, 68.3, 120.9, 129.1, 136.0, 137.8, 138.8, 153.8, 170.8, 191.4. UV λ_{max} = 282 nm (hexane). HRMS (ESI$^+$) calcd for $C_{17}H_{24}O_3$ $[M + H]^+$ 277.17982, found 277.17888.

A solution of **25** in CH_2Cl_2 (25 mL) was saponified with KOH/MeOH (2.3 mL, 10 wt %/v) at 0 °C for 2 h. The product was washed with water, dried, and concentrated to give (3*S*,6*S*)-3-hydroxy-α-ionylideneacetaldehyde (**16**) (1.00 g, 4.27 mmol; 42%). The second fraction was fully characterized as (3*R*,6*R*)-3-hydroxy-α-ionylideneacetaldehyde (**15**) (1.00 g, 4.27 mmol, 42%). The optical purities of **15** (er 97:3) and **16** (er 97:3) were established by chiral HPLC. The absolute configurations of **15** and **16** were assigned by comparison of their ^{1}H NMR and CD spectra with those of a C_{18}-ketone that was prepared by oxidative cleavage of naturally occurring (3*R*,3′*R*,6′*R*)-lutein (**1**); details are described later in this section.

(3*R*,6*R*)-3-Hydroxy-α-ionylideneacetaldehyde (15). ^{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 (ESI$^+$) calcd for $C_{15}H_{22}O_2$ $[M + H]^+$ 235.16926, found 235.17023, and $[(M + H) - H_2O]^+$ 217.15886. CD: 281 nm (+18 mdeg), 242 nm (−1.3 mdeg).

(3*S*,6*S*)-3-Hydroxy-α-ionylideneacetaldehyde (16). ^{1}H NMR and ^{13}C NMR, UV, and MS spectra of **16** were identical with those of **15**. CD: 281 nm (−13 mdeg), 242 nm (+1.0 mdeg).

Enzyme-Mediated Acylation of (3,6-*cis*,7*E*,9*E*)-3-Hydroxy-α-ionylideneacetaldehydes 17 + 18 with Lipase AK (*Pseudomonas fluorescens*). To a solution of (3,6-*cis*)-hydroxyaldehydes **17** + **18** (1.68 g, 7.17 mmol) in 15 mL of pentane were added 1.10 g of lipase AK (*Pseudomonas fluorescens*) and vinyl acetate (2.00 mL, 1.87 g, 21.72 mmol). The mixture was refluxed (35−36 °C) under N_2, and the course of the acylation was monitored by chiral HPLC. After 50 h, the product was filtered through Celite, and the filtrate was concentrated to give a yellow oil (2.0 g). Column chromatography (hexane/ethyl acetate, 98:2 to 85:15) of the product gave two major fractions. The first fraction was identified as (3*S*,6*R*)-3-acetoxy-α-ionylideneacetaldehyde (**26**) (0.85 g, 3.08 mmol; 43%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (s, 3H), 1.02 (s, 3H), 1.52 (dd, J = 23.0, 13.0, 1H), 1.66 (t, J = 1.51, 3H), 1.73 (dd, J = 13.0, 6.8, 1H), 2.08 (s, 3H), 2.27 (d, J = 1.0, 3H), 2.31 (d, J = 9.0, 1H), 5.35 (m, 1H), 5.51 (s, 1H), 5.95 (d, J = 8.0, 1H), 6.12 (dd, J = 15.6, 9.0, 1H), 6.37 (d, J = 15.6, 1H), 10.13 (d, J = 8.0, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.7, 21.8, 23.0, 27.5, 29.3, 35.2, 37.0, 55.3, 70.1, 121.8, 129.6, 135.8, 138.9, 139.0, 154.4, 191.8. UV λ_{max} = 282 nm (hexane). HRMS (ESI$^+$) calcd for $C_{17}H_{24}O_3$ $[M + H]^+$ 277.17982, found 277.18092.

A solution of **26** in CH_2Cl_2 (15 mL) was hydrolyzed with KOH/MeOH (1.6 mL, 10 wt %/v) at 0 °C for 2 h. The product was washed with water, dried, and concentrated to give (3*S*,6*R*)-3-hydroxy-α-ionylideneacetaldehyde (**17**) (0.70 g, 2.99 mmol; 42%). The second fraction was fully characterized as (3*R*,6*S*)-3-hydroxy-α-ionylideneacetaldehyde (**18**) (0.68 g, 2.90 mmol, 40%). The optical purities of **17** (er 96:4) and **18** (er 96:4) were established by chiral HPLC. The absolute configurations of **17** and **18** were assigned by comparison of their ^{1}H NMR and CD spectra with those of the acid-catalyzed epimerization products of **15**.

(3*S*,6*R*)-3-Hydroxy-α-ionylideneacetaldehyde (17). ^{1}H NMR (400 MHz, CDCl3): 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). HRMS (ESI$^+$) calcd for $C_{15}H_{22}O_2$ $[M + H]^+$ 235.16926, found 235.17121, and $[(M + H) - H_2O]^+$ 217.15762. CD: 280 nm (+17.8 mdeg).

(3*R*,6*S*)-3-Hydroxy-α-ionylideneacetaldehyde (18). ^{1}H NMR and ^{13}C NMR, UV, and MS spectra were identical with those of **17**. CD: 280 nm (−7 mdeg).

Oxidative Cleavage of (3*R*,3′*R*,6′*R*)-Lutein Diacetate to (3*R*,6*R*)-3-Hydroxy-13-apo-ε-caroten-13-one (27) and (3*R*)-3-Hydroxy-β-ionone (28). Preparation of (3*R*,3′*R*,6′*R*)-Lutein Diacetate. A solution of naturally occurring (3*R*,3′*R*,6′*R*)-lutein (**1**) (2.30 g, 4.04 mmol) in 20 mL of THF was treated with pyridine (2.5 mL, 2.45 g, 30.97 mmol) and acetic anhydride (2.50 mL, 2.70 g, 26.45 mmol), and the mixtue was heated at 45 °C under N_2 for 6 h. The product was extracted with hexane, and the organic solution was washed sequentially with aqueous HCl (5%, v/v), saturated $NaHCO_3$ solution, and water. After drying, the organic solution was concentrated to give a red solid that was purified by column chromatography on *n*-silica (hexane/acetone, from 90:10 to 70:30) to yield (3*R*,3′*R*,6′*R*)-lutein diacetate (2.35 g, 3.60 mmol; 89%). Mp 154−155 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86 (s, 3H), 0.98 (s, 3H), 1.06 (s, 6H), 1.09 (s, 3H), 1.44 (dd, J = 13.9, 5.5, 1H), 1.57 (t, J = 12.3, 1H), 1.63 (s, 3H), 1.71 (s, 3H), 1.76 (m, 1H), 1.83 (dd, J = 13.9, 6.0, 1H), 1.89 (s, 3H), 1.95 (s, 6H), 2.00 (s, 3H), 2.03 (s, 3H), 2.10 (d, J = 16.1, 8.5, 1H), 2.39 (d, J = 9.3, 1H), 2.45 (d, J = 5.5, 1H), 5.04 (m, 1H), 5.30 (s, 1H), 5.41 (dd, J = 15.5, 9.8, 1H), 5.47 (m, 1H), 6.10 (m, 2H), 6.15 (m, 3H), 6.27 (m, 2H), 6.34 (m, 2H), 6.61 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.7, 13.0, 21.4, 22.9, 25.2, 28.4, 28.9, 29.9, 33.3, 36.6, 38.3, 39.4, 43.9, 54.8, 68.3, 68.8, 119.8, 124.7, 124.8, 125.2, 125.5, 128.2, 130.0, 130.9, 131.4, 132.5, 134.9, 135.5, 136.4, 137.5, 137.7, 138.6, 140.4, 170.8. UV−vis λ_{max} = 444 nm (ethanol). HRMS (ESI^+) calcd for $C_{44}H_{60}O_4$ 652.44861, found 652.43484.

Oxidative Cleavage of (3*R*,3′*R*,6′*R*)-Lutein Diacetate. A solution of (3*R*,3′*R*,6′*R*)-lutein diacetate (1.00 g, 1.53 mmol) in ethyl acetate (30 mL) at 0 °C was treated with a 70% solution of TBHP in water (2.7 mL, 2.4 g 70% ≈ 1.7 g, 19 mmol) under N_2. Household bleach containing 5.25% NaOCl (8.8 g, 0.46 g NaOCl, 6.2 mmol) was added in 20 min at 0 °C, and the mixture was stirred at rt for 3 h. The organic solution was washed with water and concentrated, and the residue was dissolved in CH_2Cl_2 (20 mL) and saponified with KOH/MeOH (20 mL, 10%, wt/v) at rt for 2 h. The CH_2Cl_2 layer was washed with water, dried, and concentrated to give an orange solid (1.2 g). Column chromatography of the product on *n*-silica (hexane/acetone, from 95:5 to 70:30) gave unreacted **1** (0.69 g, 1.22 mmol; 80%) and a fraction that was shown by HPLC to consist of two major products. These were separated by semipreparative HPLC and identified as (3*R*,6*R*)-3-hydroxy-13-apo-ε-caroten-13-one (**27**) and (*3R*)-3-hydroxy-β-ionone (**28**).

(3*R*,6*R*)-3-Hydroxy-13-apo-ε-caroten-13-one (27). ^{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). HRMS (ESI^+) calcd for $C_{18}H_{26}O_2$ $[M + H]^+$ 275.20056, found 275.19999.

(3*R*)-3-Hydroxy-β-ionone (28). ^{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). HRMS (ESI^+) calcd for $C_{13}H_{20}O_2$ $[M + H]^+$ 209.15361, found 209.151363. CD: 310 nm (+2.98 mdeg), 270 nm (−2.87 mdeg).

Epimerization of (3*R*,6*R*)-3-Hydroxy-α-ionylideneacetaldehydes (15) to (3*S*,6*R*)-3-Hydroxy-α-ionylideneacetaldehydes (17). To a solution of **15** (3.2 mg, 0.014 mmol; er 97:3) in acetone (1 mL) and H_2O (0.5 mL) was added 0.12 mL of 0.1 N HCl, and the mixture was stirred at rt. After 28 h, HPLC showed that equilibrium had been established between hydroxyaldehydes **15** (32%) and **17** (68%). After removal of water and solvent evaporation, the hydroxyaldehydes were separated by semipreparative HPLC and characterized by ^{1}H NMR and CD as **15** and **17**.

Epimerization of (3*S*,6*R*)-3-Hydroxy-α-ionylideneacetaldehydes (17) to (3*R*,6*R*)-3-Hydroxy-α-ionylideneacetaldehydes (15). A solution of **17** (4.7 mg, 0.020 mmol; er 96:4) in acetone (1 mL) and H_2O (0.5 mL) was epimerized with 0.24 mL of 0.1 N HCl similarly to the previous experiment. After 28 h, HPLC showed an equilibrium between hydroxyaldehydes **15** (31%) and **17** (69%). These hydroxyaldehydes were separated by semipreparative HPLC and characterized by ^{1}H NMR and CD as **15** and **17**.

General Procedure for the Synthesis of C_{25}-Hydroxy-apocarotenals 6−9. Synthesis of (3*R*,6*R*)-3-Hydroxy-12′-apo-ε-caroten-12′-al (6). A solution of (3*R*,6*R*)-3-hydroxy-α-ionylideneacetaldehyde (**15**) (0.25 g, 1.1 mmol) in MeOH (3 mL) was treated with the protected Wittig salt **14** (0.82 g, 1.66 mmol) in MeOH (2 mL) at rt under N_2. One milliliter of a freshly prepared 0.42 M solution of NaOMe (0.42 mmol) in MeOH was added, and the mixture was stirred at rt for 4 h. The product was diluted with CH_2Cl_2, washed with water, and concentrated to give a red solid (1.30 g). The solids were dissolved in acetone (4 mL) and water (1 mL) and stirred with 0.3 N HCl (75 μL) at rt for 1 h under N_2. The product was extracted with CH_2Cl_2 and sequentially washed with a saturated solution of $NaHCO_3$ and water, dried, and concentrated to give a red oil. The crude product was refluxed in EtOAc in the presence of catalytic amounts of $Pd(OAc)_2$ for 2 h; in a simplified procedure, this step was omitted. After solvent evaporation, column chromatography (hexane/ethyl acetate, 95:5 to 80:20) gave (all-*E*,3*R*,6*R*)-3-hydroxy-12′-apo-ε-caroten-12′-al (**6**) (0.34 g, 0.93 mmol; 85%, er 97:3) as a red oil.

Following the above procedure, (3*S*,6*S*)-3-hydroxy-12′-apo-ε-caroten-12′-al (**7**, 61%, er 97:3), (3*S*,6*R*)-3-hydroxy-12′-apo-ε-caroten-12′-al (**8**, 53%, er 96:4), and (3*R*,6*S*)-3-hydroxy-12′-apo-ε-caroten-12′-al (**9**, 69%, er 96:4) were similarly prepared.

(3*R*,6*R*)-3-Hydroxy-12′-apo-ε-caroten-12′-al (6). ^{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). HRMS (ESI^+) calcd for $C_{25}H_{34}O_2$ $[M + H]^+$ 367.26316, found 367.26098, and $[(M + H) - H_2O]^+$ 349.25053. CD: 292 nm (+5.82 mdeg), 236 nm (+5.32 mdeg), 212 nm (+4.54 mdeg).

(3*S*,6*S*)-3-Hydroxy-12′-apo-ε-caroten-12′-al (7). ^{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). 13C 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). HRMS (ESI^+) calcd for $C_{25}H_{34}O_2$ $[M + H]^+$ 367.26316, found 367.26242, and $[(M + H) - H_2O]^+$ 349.25188. CD: 292 nm (−4.32 mdeg), 238 nm (−4.86 mdeg), 210 nm (−4.72 mdeg).

(3*S*,6*R*)-3-Hydroxy-12′-apo-ε-caroten-12′-al (8). ^{1}H 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). HRMS (ESI$^+$) calcd for $C_{25}H_{34}O_2$ [M + H]$^+$ 367.26316, found 367.26519, and [(M + H) − H_2O]$^+$ 349.25091. CD: 402 nm (+3.73 mdeg), 235 nm (+8.57 mdeg).

(3*R*,6*S*)-3-Hydroxy-12′-apo-ε-caroten-12′-al (9). ^{1}H 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). HRMS (ESI$^+$) calcd for $C_{25}H_{34}O_2$ [M + H]$^+$ 367.26316, found 367.26380, and [(M + H) − H_2O]$^+$ 349.25028. CD: 406 nm (−4.32 mdeg), 235 nm (−11.23 mdeg).

General Procedure for the Synthesis of Luteins 1−4. Synthesis of (3*R*,3′*R*,6′*R*)-Lutein (1). A solution of (3*R*,6*R*)-3-hydroxy-12′-apo-ε-caroten-12′-al (**6**) (0.26 g, 0.70 mmol) [mixture of (all-*E*)- and (11*Z*)-isomers] and (3*R*)-3-hydroxy-(β-ionylideneethyl)-triphenylphosphonium chloride (**5**) (0.41 g, 0.79 mmol) in CH_2Cl_2 (5 mL) was cooled to −5 °C under N_2. A solution of KOH (0.13 g, 2.32 mmol) in H_2O (0.5 mL) was added, and the mixture was stirred for 30 min at −5 °C and 3 h at rt. The product was diluted with CH_2Cl_2, washed with water, dried, and concentrated to give 1.0 g of a red oil. The crude product was thermally isomerized in a refluxing solution of EtOAc for 4 h under N_2. After solvent evaporation, the product was purified by column chromatography (hexane/ethyl acetate, from 90:10 to 50:50) followed by crystallization (hexane/acetone = 4:1) to yield (3*R*,3′*R*,6′*R*)-lutein (**1**) (0.296 g, 0.52 mmol; 74%, dr 99:1) as red solids.

Following the above procedure, (3*R*,3′*S*,6′*S*)-lutein (**2**, 82%, dr 99:1), (3*R*,3′*S*,6′*R*)-lutein or 3′-epilutein (**3**, 85%, dr 96:4), and (3*R*,3′*R*,6′*S*)-lutein (**4**, 80%, 94% de) were similarly prepared.

(3*R*,3′*R*,6′*R*)-Lutein (1). Mp 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$^+$) calcd for $C_{40}H_{56}O_2$ 568.42748, found 568.41896, and [(M − H_2O) + H]$^+$ 551.41697. CD: 284 nm (−1.26 mdeg), 246 nm (+2.67 mdeg), 212 nm (+3.75 mdeg). The CD spectra of **1** was identical with that of the naturally occurring (3*R*,3′*R*,6′*R*)-lutein isolated from a saponified extracts of marigold flowers.

(3*R*,3′*S*,6′*S*)-Lutein (2). Mp 128−130 °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 = 13.2, 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$^+$) calcd for $C_{40}H_{56}O_2$ 568.42748, found 568.42135, and [(M − H_2O) + H]$^+$ 551.41944. CD: 272 nm (−6.19 mdeg), 238 nm (+0.41 mdeg), 214 nm (−4.73 mdeg).

(3*R*,3′*S*,6′*R*)-Lutein (3). Mp 145−146 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86 (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$^+$) calcd for $C_{40}H_{56}O_2$ 568.42748, found 568.41427, and [(M − H_2O) + H]$^+$ 551.41547. CD: 333 nm (+1.79 mdeg), 280 nm (−2.71 mdeg), 242 nm (+4.92 mdeg).

(3*R*,3′*R*,6′*S*)-Lutein (4). Mp 114−116 °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$^+$) calcd for $C_{40}H_{56}O_2$ 568.42748, found 568.41731, and [(M − H_2O) + H]$^+$ 551.41869. CD: 328 nm (−2.08 mdeg), 266 nm (−5.03 mdeg), 238 nm (−5.78 mdeg).

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Supporting Information Available: One-pot reduction of **23a** to hydroxyaldehydes **15**−**18** and reduction of **23a** to hydroxynitriles **19**− **22** with (*R*)-2-methyl-CBS-oxazaborolidine. Copies of ^{1}H and ^{13}C NMR spectra of compounds **1**−**4**, **6**−**9**, **15**−**28**, and (3*R*,3′*R*,6′*R*)-lutein diacetate. Copies of CD spectra of naturally occurring (3*R*,3′*R*,6′*R*)-lutein and compounds **1**−**4**, **6**−**9**, and **15**−**18**. Table 1S: detailed reduction of ketonitrile **23a** to hydroxynitriles **19**−**22**. Table 2S: details of HPLC (chiral, normal phase) separations and copies of HPLC traces for relevant compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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