Synthesis of (3*R*,3′*R*)-Zeaxanthin and Its *meso*-Stereoisomer from (3*R*,3′*R*,6′*R*)-Lutein via (3*R*)-3′,4′-Anhydrolutein

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Abstract: A process has been developed for the partial synthesis of (3*R*,3′*R*)-zeaxanthin and (3*R*,3′*S*; *meso*)-zeaxanthin from commercially available (3*R*,3′*R*,6′*R*)-lutein. This involves the regioselective hydroboration of a dehydration product of lutein, namely (3*R*)-3′,4′-didehydro-β,β-caroten-3-ol [(3*R*)-3′,4′-anhydrolutein], to yield a mixture of (3*R*,3′*R*)-zeaxanthin and (3*R*,3′*S*; *meso*)-zeaxanthin followed by separation of these carotenoids by enzyme-mediated acylation. (3*R*,3′*R*,6′*R*)-Lutein, (3*R*,3′*R*)-zeaxanthin and its *meso*-isomer accumulate in human ocular tissues and have been implicated in the prevention of age-related macular degeneration (AMD).

Key words: optically active hydroxycarotenoids, partial synthesis, chiral resolution, regioselective hydroboration, highly conjugated polyenes

(3*R*,3′*R*,6′*R*)-Lutein (**1**) and (3*R*,3′*R*)-zeaxanthin (**2**) are two dietary carotenoids that are present in most fruits and vegetables commonly consumed in the US.[1] These carotenoids accumulate in the human plasma[2,3] and ocular tissues [macula, retinal pigment epithelium (RPE), ciliary body, iris, lens][4,5] and have been implicated in the prevention of age-related macular degeneration (AMD) which is the leading cause of blindness in the US and Western World.[6–9] There are also two stereoisomers of **2**, namely (3*R*,3′*S*; *meso*)-zeaxanthin (**3**) and (3*S*,3′*S*)-zeaxanthin; these carotenoids are not of dietary origin. However, *meso*-zeaxanthin, which is absent from human plasma, has been found in nearly all human ocular tissues.[6,10] This carotenoid is presumably formed in the human eye tissues as a consequence of metabolic transformation of dietary **1**.[4,11] Among the seven stereoisomers of **1**, only (3*R*,3′*S*,6′*R*)-lutein (3′-epilutein, **4**) has been detected in the human plasma and tissues.[2,3,12] The chemical structures of these carotenoids are shown in Figure 1.

In view of the potential therapeutic application of **1**, **2**, and **3**, the industrial production of these carotenoids has received considerable attention. Due to its challenging total synthesis,[13,14] **1** is isolated from saponified extracts of marigold flowers (*Tagete erecta, variety orangeade*) and is commercially available as a nutritional supplement.[15] Although **2** is widely distributed in Nature, its concentration in most readily available natural products is not sufficiently high for commercial production by extraction and isolation. Therefore, several lengthy multistep processes have been developed for the total synthesis of this caro-

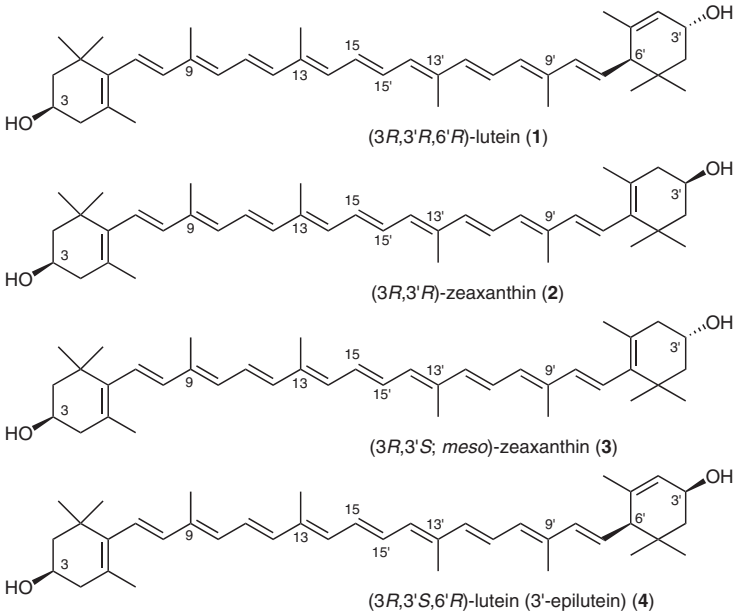

Figure 1 Chemical structures of dietary lutein (**1**) and zeaxanthin (**2**), and their metabolites

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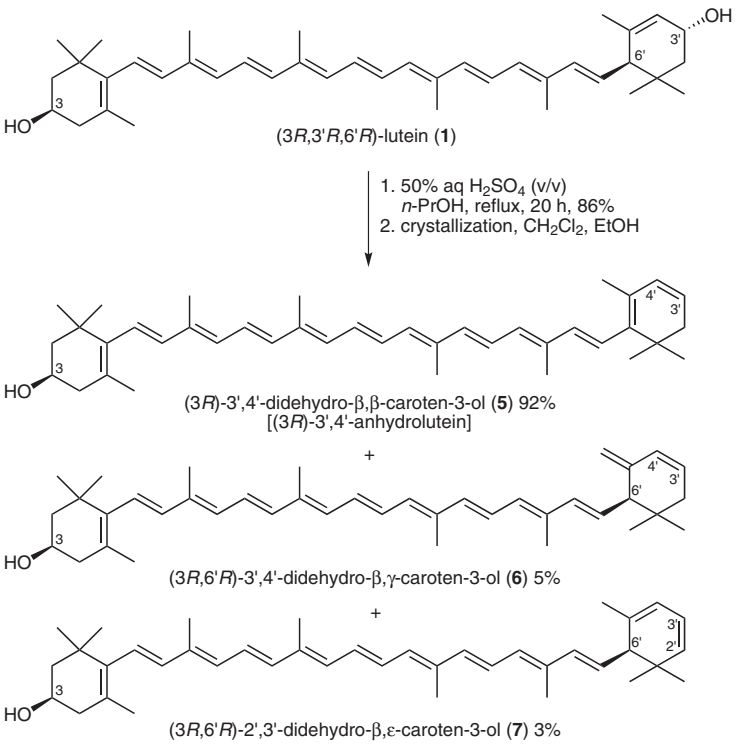
tenoid.[16–19] There are also several processes that convert the commercially available **1** or crude saponified extracts of marigold flowers by base-catalyzed isomerization into optically inactive **3**[20,21] or a racemic mixture of zeaxanthin;[22,23] however, due to the low overall yield and the fact that the racemic mixture of (3*RS*,3′*RS*)-zeaxanthin is not resolved, these processes do not provide an attractive route to **2** and **3**. To circumvent these problems, we reported an efficient process in which **1** was first converted into 3′-epilutein (**4**) by acid-catalyzed epimerization and the latter was then transformed to **2** by base-catalyzed isomerization.[24]

The author now wishes to report an alternative method for the transformation of dietary **1** to **2** and **3**. These carotenoids are initially prepared as a diastereomeric mixture that is subsequently separated into individual compounds with the aim to provide access to both of these important nutrients.

The strategy was to take advantage of a readily accessible dehydration product of **1**, namely (3*R*)-3′,4′-didehydro-β,β-caroten-3-ol [(3*R*)-3′,4′-anhydrolutein, **5**], as the starting material for the preparation of **2** and **3**. We have previously prepared **5** by acid-catalyzed dehydration of **1** in *n*-propanol under reflux, followed by crystallization, as shown in Scheme 1.[25] This reaction resulted in the formation of two other minor products in addition to **5**; these have been previously identified as (3*R*,6′*R*)-3′,4′-didehydro-β,γ-caroten-3-ol (**6**) and (3*R*,6′*R*)-2′,3′-didehydro-β,ε-caroten-3-ol (**7**). These carotenoids were initially obtained in the ratio of **5/6/7** = 82:10:6, along with 2% of **2** that was present in **1** and remained unreacted under the reaction conditions. The second crystallization of this mixture from dichloromethane and ethanol resulted in the removal of **2** and afforded a mixture that consisted of mainly **5** (92%) and minor quantities of **6** (5%) and **7** (3%).

The transformation of **5** to a mixture of **2** and **3** was accomplished by regioselective hydroboration with a wide range of reagents, followed by crystallization. These reagents included borane–tetrahydrofuran complex (BH_3·THF), borane–dimethyl sulfide complex (BH_3·SMe_2), (–)-isopinocampheylborane–*N*,*N*,*N*′,*N*′-tetramethylethylenediamine complex [(*R*)-Alpine-Boramine™], and (*S*)-Alpine-Boramine™. As shown in Table 1, in addition to the desired products, this reaction also resulted in the formation of two side products that were isolated and identified from their NMR, UV/Vis, and MS data as (3*R*,4′*RS*)-β,β-carotene-3,4′-diol (**8**) and (3*R*,6′*RS*)-β,ε-caroten-3-ol (α-cryptoxanthin, **9**). These side products were readily removed by crystallization of the crude product with dichloromethane and hexane and did not contaminate the final product. While **8** is a regioisomer of **2** and **3** and its formation from **5** by hydroboration would be expected, the mechanism by which **9** is formed is not clear at present. This side product appears to have been formed by hydrogenation of **5** accompanied by double-bond isomerization. It is interesting to point out that the hydroboration of double bonds of terpenes followed by protonolysis with an organic acid has been well documented by H. C. Brown to result in hydrogenation of these compounds;[26–28] however, it is not clear how **5** could undergo hydrogenation and double-bond isomerization to **9** by hydroboration in the absence of acids.



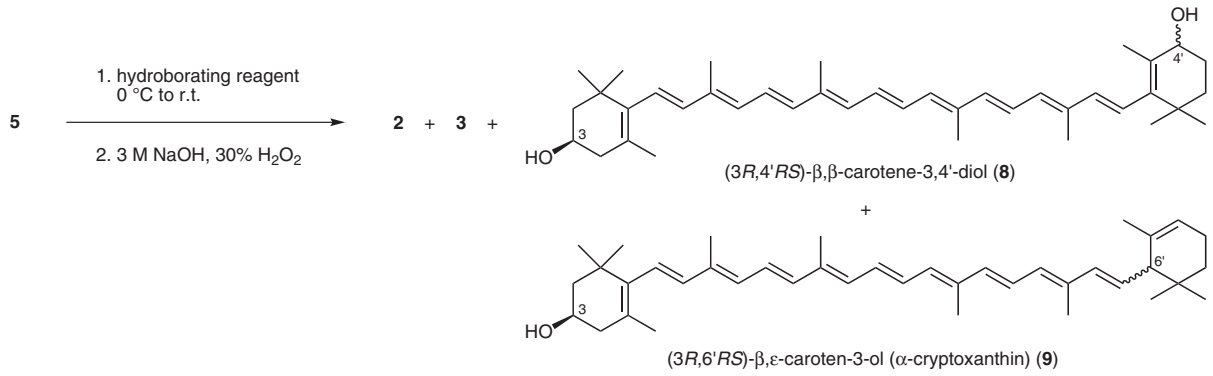
Scheme 1 Dehydration of **1** according to the method of Khachik and co-workers[25]

The yields in Table 1 are based on the isolated yield of the purified mixture of (3*R*,3′*R*)-zeaxanthin (**2**) and (3*R*,3′*S*; *meso*)-zeaxanthin (**3**) that was obtained after crystallization or chromatography. The hydroboration reaction in all cases resulted in complete conversion of **5** into the products and the best results were obtained with borane in ether solvents (entries 1–3) prepared from sodium borohydride and iodomethane at room temperature. With the exception of entry 4, all reactions in Table 1 were carried out at room temperature. When the reaction was carried out with a commercial solution (1 M) of BH_3·THF (Table 1, entry 4), the regioselectivity of hydroboration was considerably reduced and **8** was formed to a greater extent. This was also the case when the reactions were carried out at 0 °C with BH_3·THF prepared from sodium borohydride and iodomethane (data not shown). The reaction of **5** with BH_3·SMe_2 in tetrahydrofuran also resulted in poor regioselectivity. Particularly interesting were the hydroborations of **5** with (*R*)- and (*S*)-Alpine-Boramine™ that are well-established reagents for the stereospecific hydroboration of alkenes.[29] With (*R*)-Alpine-Boramine™, **2** was obtained in 54% diastereomeric excess (de), while (*S*)-Alpine-Boramine™ did not show any significant diastereoselectivity and the de of **3** was only 14%. The diastereomeric ratio of **2** and **3** was determined by chiral HPLC (eluent B) according to our published method.[10]

In the following step, the diastereomeric mixture of **2** and **3** was separated by irreversible enzyme-mediated acylation of the mixture with immobilized lipase PS (*Pseudomonas cepacia*) or lipase AK (*Pseudomonas fluorescens*) in the presence of vinyl acetate. Within the first 24 hours, these carotenoids initially underwent monoacylation at room temperature to give a mixture of (3*R*,3′*R*)-zeaxanthin-3-acetate (**10**) and (3*R*,3′*S*)-zeaxanthin-3-acetate (**11**), that was accompanied by minor quantities of (3*R*,3′*R*)-zeaxanthin-3,3′-diacetate (**12**), as shown in Scheme 2. This was clearly shown by monitoring the course of the reaction by chiral HPLC (eluent B) that revealed the gradual conversion of **2** and **3** into their corresponding monoacetates.

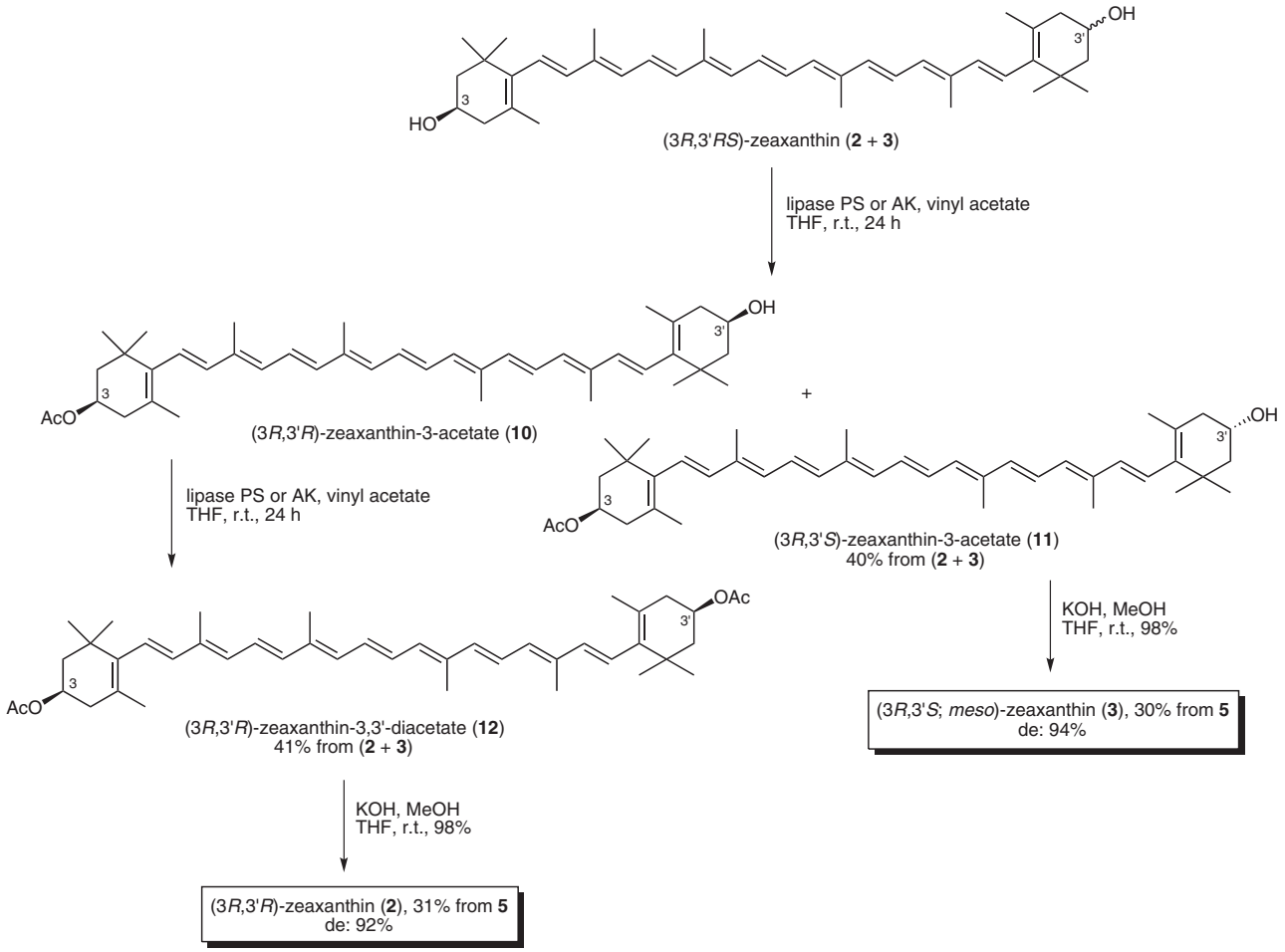
When the reaction was allowed to proceed for an additional 24 hours at room temperature, **10** was acylated to **12**, while **11** remained unchanged. The progress of this reaction was monitored by HPLC employing eluent C that separates the zeaxanthin monoacetates. The overall reaction time was substantially reduced when the enzyme-mediated acylation was carried out at 40–45 °C. After the enzyme was removed, the crude products were separated by column chromatography on silica gel (hexane–

Table 1 Transformation of **5** to **2** and **3** by Regioselective Hydroboration



Entry	Reagent[a]	Product distribution[b] (%) (**2** + **3**)/**8**/**9**	Isolated yield[c] (%) (**2** + **3**)
1	BH_3·THF	92:2:6	75
2	BH_3·DME	85:5:10	57
3	BH_3·TBME·diglyme	88:4:8	55
4	BH_3·THF[d]	77:17:6	60
5	BH_3·SMe_2·THF	75:23:2	55
6	(*R*)-Alpine-Boramine™·THF	85:9:6	40[e,f]
7	(*S*)-Alpine-Boramine™·THF	80:13:7	40[e,g]

[a] The hydroborating reagents in entries 1–3 were prepared from $NaBH_4$ and MeI.
[b] The relative distribution of the products was determined by HPLC (eluent A).
[c] Isolated yield of **2** + **3** after crystallization or chromatography.
[d] Reaction was carried out with a commercial solution of BH_3·THF at 0 °C.
[e] Diastereomeric ratio was determined by chiral HPLC (eluent B).
[f] Ratio of **2**/**3** = 77:23 (54% de).
[g] Ratio of **2**/**3** = 43:57.



Scheme 2 Separation of **2** and **3** by irreversible enzyme-mediated acylation

acetone, 95:5 to 90:10). Due to the difference in their solubility and chromatographic properties, **12** eluted from the column almost immediately whereas **11** was retained and eluted much later from the column. The alkaline hydrolysis of these individually pure di- and monoacetates of zeaxanthin was carried out in an alcoholic solution of potassium hydroxide to afford **2** and **3** in 92% de and 94% de, respectively. Similar results were also obtained with lipase AK (*Pseudomonas fluorescens*) (data not shown). The structures of **2** and **3** were established by comparison of their HPLC retention times and UV/Vis spectra obtained by photodiode array detection on a chiral HPLC column with those of standard reference samples of these carotenoids that were prepared earlier in our laboratory. It should be noted that **2** and **3** exhibit identical ^{1}H and ^{13}C NMR spectra despite their diastereomeric relationship. This has been well documented in the literature and has been attributed to the remote stereogenic centers at the C-3 and C-3′ positions.[17] Consequently, the NMR spectra of these carotenoids were merely obtained to confirm the structure of zeaxanthin, and chiral HPLC was employed to determine their diastereomeric ratios.

In summary, a method has been developed that allows the transformation of commercially available (3*R*,3′*R*,6′*R*)-lutein (**1**) into a mixture of **2** and **3** via (3*R*)-3′,4′-didehydro-β,β-caroten-3-ol [(3*R*)-3′,4′-anhydrolutein, **5**] which is a readily accessible dehydration product of **1**. The overall yields of individually purified **2** and **3** from **5** were 31% and 30%, respectively. We have previously shown that **5** also serves as a key intermediate in the partial synthesis of (3*R*)-β-cryptoxanthin which is a provitamin A carotenoid with important biological activities.[25] Therefore, this process provides yet another application for (3*R*)-3′,4′-anhydrolutein (**5**). As pointed out earlier, **2** and **3** are found in human ocular tissues and have been implicated in the prevention of age-related macular degeneration. Therefore, the purified mixture of these carotenoids can be directly used as a nutritional supplement; however, for the first time, a methodology has been developed that allows the separation of (3*R*,3′*R*)-zeaxanthin (**2**) from its *meso*-stereoisomer **3** by enzyme-mediated acylation in high diastereomeric excess.

Borane–ether complex solutions were prepared fresh from $NaBH_4$ and MeI in ether solvents by a slight modification of a published method.[30] All other chemicals and reagents including commercial solutions of BH_3·THF (1 M), BH_3·SMe_2, (*R*)-Alpine-Boramine™, and (*S*)-Alpine-Boramine™ were obtained from Aldrich Chemical Co. (St. Louis, MO). Lipase PS (*Pseudomonas cepacia*) and lipase AK (*Pseudomonas fluorescens*) were obtained from Amano Enzyme USA (Lombard, IL); these enzymes were immobilized similar

to a published procedure.[31] Column chromatographic purifications were carried out on silica gel (Merck 100–200 mesh). ^{1}H NMR spectra were recorded on a Bruker DRX-400 spectrometer (400 MHz) with $CDCl_3$ (7.27 ppm) as internal standard. ^{1}H noise-decoupled ^{13}C spectra were recorded on a Bruker DRX-400 instrument at 100 MHz with $CDCl_3$ (77.0 ppm) as internal standard. High-resolution mass spectra (HRMS) were obtained on a JEOL AccuTOF CS mass spectrometer (ion source: ESI, needle voltage: 2300 V, flow rate: 100 μL/min, desolvation chamber temperature: 250 °C, data acquisition time: 2 min). The course of hydroboration reactions was monitored by HPLC (eluent A) on a silica-based nitrile-bonded column (25-cm length × 4.6-mm i.d., 5-μm spherical particle; Waters Corporation, Milford, MA). The column was protected with a Brownlee nitrile-bonded guard cartridge (3-cm length × 4.6-mm i.d., 5-μm particle size). Eluent A consisted of an isocratic mixture of hexane (75%), CH_2Cl_2 (25%), and MeOH (1%). The column flow rate was 0.7 mL/min and the separations were monitored at 454 nm for zeaxanthins (**2** + **3**), (3*R*,4′*RS*)-β,β-carotene-3,4′-diol (**8**), and α-cryptoxanthin (**9**), and at 466 nm for (3*R*)-3′,4′-anhydrolutein (**5**). In the order of elution, the HPLC retention times were: **9** (10.37 min), **5** (10.74 min), **8** (28.71 min), and **2** + **3** (coeluting peaks at 37.68 min). The optical purity of **2** and **3** was assessed by chiral HPLC (eluent B) on a Chiralpak® AD column (25-cm length × 4.6-mm i.d.) from Chiral Technologies (Exton, PA). The column packing consisted of amylose tris(3,5-dimethylphenylcarbamate) coated on 10-μm silica gel substrate and the column was protected with a silica gel guard cartridge (3-cm length × 4.6-mm i.d., 5-μm particle size). For eluent B, a two-pump system with a combination of isocratic and gradient HPLC was employed that separated the stereoisomers of zeaxanthin, monitored at 450 nm. Pump 1 pumped a mixture of hexane (95%) and *i*-PrOH (5%), and pump 2 pumped a mixture of hexane (85%) and *i*-PrOH (15%). At time zero, 95% solvents from pump 1 and 5% solvents from pump 2 were pumped isocratically for 10 min. After 10 min, a linear gradient was run for 15 min during which the solvents from pump 2 were linearly increased from 5% to 40% while those of pump 1 were reduced from 95% to 60%. At the end of each run, the column was re-equilibrated under the original isocratic conditions for 20 min. It should be noted that in addition to the separation of **2** and **3**, this HPLC system could also separate (3*S*,3′*S*)-zeaxanthin; however, this stereoisomer of zeaxanthin is not formed according to the process described here. Semipreparative HPLC separation for the isolation of **8** and **9** was carried out on a silica-based nitrile-bonded column (25-cm length × 10-mm i.d., 10-μm spherical particle; Waters Corporation, Milford, MA) employing eluent A at a flow rate of 3 mL/min. The column was protected with a Brownlee nitrile-bonded guard cartridge (3-cm length × 4.6-mm i.d., 5-μm particle size). The enzymatic acylation of the diastereomeric mixture of **2** and **3** was initially monitored by chiral HPLC employing eluent B until both zeaxanthins were monoacylated to (3*R*,3′*R*)-zeaxanthin-3-acetate (**10**) and (3*R*,3′*S*)-zeaxanthin-3-acetate (**11**), respectively. To monitor the enzyme-mediated acylation of **10** to (3*R*,3′*R*)-zeaxanthin-3,3′-diacetate (**12**), an isocratic mixture (eluent C) of hexane (95%) and *i*-BuOH (5%) was used with the Chiralpak® AD column at a flow rate of 0.7 mL/min. Under these conditions, the monoacyl esters were separated and the course of the enzyme-mediated acylation could be easily monitored.

Purification of (3*R*)-3′,4′-Anhydrolutein (5)

A crude mixture (20 g) of (3*R*)-3′,4′-anhydrolutein (**5**, 82%), **6** (10%), **7** (6%), and **2** (2%), prepared by dehydration of (3*R*,3′*R*,6′*R*)-lutein (**1**) according to an earlier publication,[25] was dissolved in CH_2Cl_2 (100 mL) and EtOH (200 mL) was added. The mixture was stirred at r.t. for 1 h and kept at 0 °C for 4 h. The dark red crystals (14 g) were collected by filtration and dried under high vacuum overnight. The product was shown by HPLC (conditions described in Ref.[25]) to consist of **5** (92%), **6** (5%), and **7** (3%). The ^{1}H and ^{13}C NMR data of the product were identical with those of **5** reported in our earlier publication.[32]

Hydroboration of (3*R*)-3′,4′-Anhydrolutein (5); Typical Procedure

The hydroboration of **5** with BH_3·THF is described here as a typical procedure for the hydroborations with borane–ether complex solutions (Table 1, entries 1–3).

(3*R*)-3′,4′-Anhydrolutein (**5**; 5 g, 9.08 mmol) and $NaBH_4$ (0.72 g, 19.02 mmol) were transferred into a 250-mL flask equipped with an argon inlet, a thermometer, a mechanical stirrer, and an addition funnel. Anhyd THF (130 mL) was added and the mixture was stirred under argon. The flask was immersed in a cold-water bath (~10–15 °C) and a soln of MeI (1.2 mL, 2.736 g, 19.28 mmol) in anhyd THF (10 mL) was added dropwise at r.t. in 10 min during which gas evolution began and a thick, dark red paste was formed. After 2 h, no detectable amount of **5** was shown by HPLC (eluent A) to be present. The mixture was cooled to –10 °C, MeOH (15 mL) was added dropwise, and the mixture was stirred until all the solids were dissolved. This was followed by sequential and slow addition of 3 M NaOH (15 mL) and 30% H_2O_2 (15 mL) while maintaining the temperature at –10 °C. The mixture was allowed to warm to r.t. and stirred for 1 h; during this period, the temperature rose to 29 °C and then dropped back to r.t. The solids were removed by filtration and the filtrate was partitioned between H_2O (150 mL) and CH_2Cl_2 (150 mL). The organic layer was washed with H_2O (2 × 100 mL), dried over Na_2SO_4, and filtered. HPLC analysis (eluent A) of the crude product showed the presence of **2** and **3** as coeluting peaks (92%), (3*R*,4′*RS*)-β,β-carotene-3,4′-diol (**8**, 2%), and α-cryptoxanthin (**9**, 6%). The solution of the crude product was concentrated under reduced pressure and the dark orange residue was crystallized from CH_2Cl_2 (100 mL) and hexane (150 mL). After the mixture was cooled at 0–5 °C for several hours, the crystals were collected by filtration and dried under high vacuum overnight to give a mixture of **2** and **3**; yield: 3.87 g (6.81 mmol, 75%); orange crystals. HPLC analysis of the product did not show the presence of any other carotenoids or impurities, and the ^{1}H and ^{13}C NMR spectra of zeaxanthins (**2** + **3**) were identical with those of **2** reported in our earlier publication.[33]

The filtrate was subjected to semipreparative HPLC (eluent A) which allowed the isolation and subsequent characterization of **8** and **9**.

(3*R*,4′*RS*)-β,β-Carotene-3,4′-diol (8)

^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.99 (s, 3 H), 1.03 (s, 3 H), 1.08 (s, 6 H), 1.46 (m, 2 H), 1.62–1.72 (m, 2 H), 1.75 (s, 3 H), 1.77 (m, 1 H), 1.85 (s, 3 H), 1.92 (m, 1 H), 1.98 (s, 12 H), 2.05 (m, 1 H), 2.39 (m, 1 H), 4.02 (br m, 2 H), 6.08–6.21 (m, 6 H), 6.27 (br d, *J* = 8 Hz, 2 H), 6.38 (d, *J* = 15 Hz, 2 H), 6.60–6.70 (m, 4 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 12.8, 18.7, 21.6, 21.8, 27.8, 28.6, 28.8, 29.1, 30.3, 34.6, 34.8, 37.2, 42.5, 48.5, 65.0, 70.3, 125.0, 125.4, 125.7, 125.8, 126.2, 130.0, 130.2, 131.3, 131.7, 132.6, 135.7, 135.8, 136.5, 137.7, 137.9, 138.5, 138.8, 141.8.

HRMS (ESI$^+$): *m*/*z* [M]$^+$ calcd for $C_{40}H_{56}O_2$: 568.42748; found: 568.42600.

UV/Vis (EtOH): λ_{max} = 452 nm (main max).

(3*R*,6′*RS*)-β,ε-Caroten-3-ol (α-Cryptoxanthin) (9)

The ^{1}H and ^{13}C NMR spectra of **9** were in agreement with our earlier published data.[25]

HRMS (ESI$^+$): *m*/*z* [M]$^+$ calcd for $C_{40}H_{56}O$: 552.43257; found: 552.43200.

UV/Vis (hexane): λ_{max} = 446 nm (main max).

Hydroboration of 5 with a Commercial Solution of BH_3·THF at 0 °C

To a stirred soln of **5** (1 g, 1.82 mmol) in THF (30 mL) at 0 °C under argon was added a 1 M soln of BH_3·THF (6.0 mL, 6.0 mmol) in 15 min. After 3 h, no detectable amount of **5** was shown by HPLC to be present. The mixture was cooled to –10 °C and was sequentially treated with MeOH (3 mL), 3 M NaOH (3 mL), and 30% H_2O_2 (3 mL), and worked up as described above. HPLC analysis (eluent A) of the crude product showed the presence of **2** and **3** as coeluting peaks (77%), **8** (17%), and **9** (6%). Purification of the product by column chromatography (hexane–acetone, 95:5 to 70:30) gave a mixture of zeaxanthins **2** and **3**; yield: 0.62 g (1.09 mmol, 60%).

Hydroboration of 5 with BH_3·SMe_2

To a soln of **5** (1 g, 1.82 mmol) in CH_2Cl_2 (30 mL) under argon was added a 2 M soln of BH_3·SMe_2 (1.9 mL, 3.8 mmol) at r.t. in 5 min. After 3 h, no detectable amount of **5** was shown by HPLC to be present. The mixture was cooled to –10 °C and was sequentially treated with MeOH (3 mL), 3 M NaOH (3 mL), and 30% H_2O_2 (3 mL), and worked up as described above. HPLC analysis (eluent A) of the crude product showed the presence of **2** and **3** as coeluting peaks (75%), **8** (23%), and **9** (2%). Purification of the product by column chromatography (hexane–acetone, 95:5 to 70:30) gave a mixture of zeaxanthins **2** and **3**; yield: 0.57 g (1.00 mmol, 55%).

Hydroboration of 5 with (–)-Isopinocampheylborane–*N*,*N*,*N*′,*N*′-Tetramethylethylenediamine Complex [(*R*)-Alpine-Boramine™]

To a soln of (*R*)-Alpine-Boramine™ (0.33 g, 0.793 mmol) in THF (5 mL) was added a soln of BF_3·OEt_2 (0.2 mL, 0.224 g, 1.58 mmol) at r.t. under argon and the mixture was stirred for 1 h. The mixture was then cooled to 0 °C and a soln of **5** (0.24 g, 0.44 mmol) in THF (5 mL) was added. After 3 h, the mixture was sequentially treated with MeOH (0.5 mL), 3 M NaOH (0.5 mL), and 30% H_2O_2 (0.5 mL), and worked up with EtOAc as described above. HPLC analysis (eluent A) of the crude product showed the presence of **2** and **3** as coeluting peaks (85%), as well as **8** (9%) and **9** (6%). Purification of the product by column chromatography (hexane–acetone, 95:5 to 70:30) gave a mixture of zeaxanthins **2** and **3**; yield: 0.100 g (0.176 mmol, 40%). Chiral HPLC analysis (eluent B) of the mixture revealed the ratio of **2/3** = 77:23, corresponding to 54% de for **2**.

Hydroboration of 5 with (+)-Isopinocampheylborane–*N*,*N*,*N*′,*N*′-Tetramethylethylenediamine Complex [(*S*)-Alpine-Boramine™]

The hydroboration of **5** (0.24 g, 0.44 mmol) with (*S*)-Alpine-Boramine™ was carried out similar to the above experiment with (*R*)-Alpine-Boramine™, to yield a mixture of **2** and **3** (80%), as well as **8** (13%) and **9** (7%). Purification of the product by column chromatography (hexane–acetone, 95:5 to 70:30) gave a mixture of **2** and **3**; yield: 0.100 g (0.176 mmol, 40%). The ratio of **2/3** = 43:57, corresponding to 14% de for **3**, was determined by chiral HPLC analysis (eluent B) of the mixture.

Separation of 2 and 3 by Enzyme-Mediated Acylation with Lipase PS (*Pseudomonas cepacia*)

A mixture of **2** and **3** (0.6 g, 1.06 mmol) in THF (20 mL) was treated with immobilized lipase PS (0.6 g) and vinyl acetate (3 mL), and the mixture was stirred at r.t. under argon. The course of the reaction was monitored by chiral HPLC (eluent B). After 24 h, HPLC showed complete conversion of **2** and **3** into (3*R*,3′*R*)-zeaxanthin-3-acetate (**10**) and (3*R*,3′*S*)-zeaxanthin-3-acetate (**11**), as well as minor quantities of (3*R*,3′*R*)-zeaxanthin-3,3′-diacetate (**12**). Stirring was continued for another 24 h and the progress of the reaction was monitored by chiral HPLC employing eluent C. When **10** was completely acylated to **12** (total of 48 h), the enzyme was removed by filtration and the solvent was evaporated under reduced pressure to give 0.70 g of a crude product. Column chromatography of the residue on silica gel (hexane–acetone, 95:5 to 90:10) gave two main fractions. The first fraction was identified as (3*R*,3′*R*)-zeaxanthin-3,3′-diacetate (**12**) and the second fraction was identified as (3*R*,3′*S*)-zeaxanthin-3-acetate (**11**).

(3*R*,3′*R*)-Zeaxanthin-3,3′-diacetate (12)

Yield: 0.28 g [0.43 mmol, 41% from (**2** + **3**)].

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.08 (s, 6 H), 1.11 (s, 6 H), 1.59 (t, *J* = 12 Hz, 2 H), 1.73 (s, 6 H), 1.77 (m, 2 H), 1.98 (d, *J* = 2 Hz, 12 H), 2.07 (s, 6 H), 2.14 (dd, *J* = 17, 8 Hz, 2 H), 2.46 (dd, *J* = 17, 5 Hz, 2 H), 5.07 (m, 2 H), 6.10 (m, 2 H), 6.13 (m, 2 H), 6.16 (d, *J* = 12 Hz, 2 H), 6.27 (br m, 2 H), 6.38 (d, *J* = 15 Hz, 2 H), 6.60–6.70 (m, 4 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 12.75, 12.82, 21.47, 21.49, 28.52, 30.00, 36.70, 38.46, 44.02, 48.43, 68.43, 124.89, 125.28, 125.62, 130.09, 131.44, 135.59, 136.47, 136.50, 137.64, 137.85, 138.67, 170.83.

HRMS (ESI$^+$): *m*/*z* [M]$^+$ calcd for $C_{44}H_{60}O_4$: 652.448612; found: 652.448200.

UV/Vis (hexane): λ_{max} = 452 nm (main max).

(3*R*,3′*S*)-Zeaxanthin-3-acetate (11)

Yield: 0.256 g [0.42 mmol, 40% from (**2** + **3**)].

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.08 (s, 9 H), 1.11 (s, 3 H), 1.49 (t, *J* = 12 Hz, 1 H), 1.59 (t, *J* = 12 Hz, 1 H), 1.73 (s, 3 H), 1.75 (s, 3 H), 1.77 (m, 2 H), 1.98 (d, *J* = 2 Hz, 12 H), 2.06 (dd, *J* = 17, 9.8 Hz, 1 H), 2.07 (s, 3 H), 2.12 (dd, *J* = 17, 9 Hz, 1 H), 2.40 (dd, *J* = 17, 6 Hz, 1 H), 2.46 (dd, *J* = 17, 6 Hz, 1 H), 4.01 (m, 1 H), 5.07 (m, 1 H), 6.12 (m, 3 H), 6.16 (m, 3 H), 6.27 (br m, 2 H), 6.38 (d, *J* = 15 Hz, 2 H), 6.60–6.70 (m, 4 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 12.75, 12.82, 21.47, 21.49, 21.62, 28.52, 28.73, 30.00, 30.25, 36.70, 38.46, 42.55, 44.02, 48.43, 65.09, 68.43, 124.89, 124.92, 125.28, 125.57, 125.62, 126.16, 130.06, 130.09, 131.31, 131.44, 132.59, 132.64, 135.59, 135.69, 136.47, 136.50, 137.56, 137.64, 137.75, 137.85, 138.50, 138.67, 170.83.

HRMS (ESI$^+$): *m*/*z* [M]$^+$ calcd for $C_{42}H_{58}O_3$: 610.438047; found: 610.438200.

UV/Vis (hexane): λ_{max} = 452 nm (main max).

(3*R*,3′*R*)-Zeaxanthin (2)

The first fraction (**12**) was dissolved in THF (10 mL) and stirred with KOH in MeOH (10% wt/v, 2 mL) for 1 h at r.t. under argon. The product was partitioned between H_2O (20 mL) and CH_2Cl_2 (20 mL). The organic layer was removed and washed with H_2O (2 × 20 mL), dried over Na_2SO_4, and concentrated to dryness to give (3*R*,3′*R*)-zeaxanthin (**2**); yield: 0.239 g (0.42 mmol, 98% from **12**). The product was shown by chiral HPLC (eluent B) to consist of **2** (96%) and (3*R*,3′*S*; *meso*)-zeaxanthin (**3**, 4%), corresponding to 92% de for **2**. The ^{1}H NMR spectrum of this fraction was in agreement with that of (3*R*,3′*R*)-zeaxanthin reported in our earlier publication.[33]

(3*R*,3′*S*; *meso*)-Zeaxanthin (3)

The second fraction (**11**) was dissolved in THF (10 mL) and stirred with KOH in MeOH (10% wt/v, 2 mL) for 1 h at r.t. under argon. The product was worked up as described above for **2** to give (3*R*,3′*S*; *meso*)-zeaxanthin (**3**); yield: 0.233 g (0.41 mmol, 98% from **11**). The product was shown by chiral HPLC (eluent B) to consist of **3** (97%) and (3*R*,3′*R*)-zeaxanthin (**2**, 3%), corresponding to 94% de for **3**. The ^{1}H NMR spectrum of this fraction was identical with that of (3*R*,3′*R*)-zeaxanthin (**2**).

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