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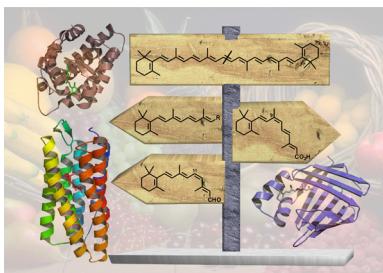
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Functions, Therapeutic Applications, and Synthesis of Retinoids and Carotenoids

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1. INTRODUCTION

Vitamin A (*all-trans*-retinol, **1**, Figure 1) is considered the most multifunctional vitamin in the human body.¹ It plays key roles in many physiological processes such as vision, reproduction, embryonic growth and development, immune competence, cell differentiation, cell proliferation and apoptosis, maintenance of epithelial tissue, and brain function.² A century after³ the role of vitamin A as dietary component required for normal growth and vision was established,⁴ vitamin A deficiency (serum vitamin A levels of <0.7 $\mu\text{mol L}^{-1}$) is still prevalent in many developing countries, and considered responsible for child (estimated as 600 000 per year) and maternal mortality.⁵ The administration of vitamin A alone⁶ has been shown to decrease preschool mortality in developing countries by 23–34% (The State of the World's Children, UNICEF, 2011).⁷ Severe vitamin A deficiency can lead to xerophthalmia and night blindness.^{5b}

β,β -Carotene (**4**, Figure 1) and a few other provitamin A carotenoids are the major dietary source for vitamin A in humans.⁸ Carotenoids are biosynthesized by all photosynthetic plants, protists, and bacteria, as well as some heterotrophic bacteria, some fungi, and some invertebrates (aphids, the two-spotted spider mite), but other animals must ingest them in the diet. Human health benefits from the physiological functions linked to carotenoid intake because these compounds⁹ prevent oxidative tissue damage, act as immunostimulants and as yolk nourishment to embryos, and are responsible for photoprotection by accumulating in light exposed tissues and scavenging reactive oxygen species (ROS).¹⁰ Carotenoids found in fruit and vegetables, such as β,β -carotene (**4**), have significant implications for nutrition.^{2d} Epidemiologic studies found that higher dietary intakes of β,β -carotene and higher blood concentrations of β,β -carotene were associated with a 20–50% decreased risk of lung cancer. However, two large clinical trials of β,β -carotene supplementation, the Alpha-Tocopherol Beta-Carotene Cancer Prevention Study and the Carotene and Retinol Efficacy Trial, were discontinued after finding increased incidence of lung cancer in heavy smokers.¹¹ Carotenoids also promote visual tuning and prevent macular degeneration.¹² The macula lutea, a central part of the retina with yellow coloration, is enriched in the xanthophylls (i.e., oxygen-containing carotenoids) lutein and zeaxanthin. Zeaxanthin and lutein reduce the risk of developing eye disease, and improve visual performance in age-related macular degeneration (AMD) and cataracts by acting as antioxidants against lipid oxidation and filtering short-wavelength visible light and protecting underlying cell layers from potential light damage.¹³

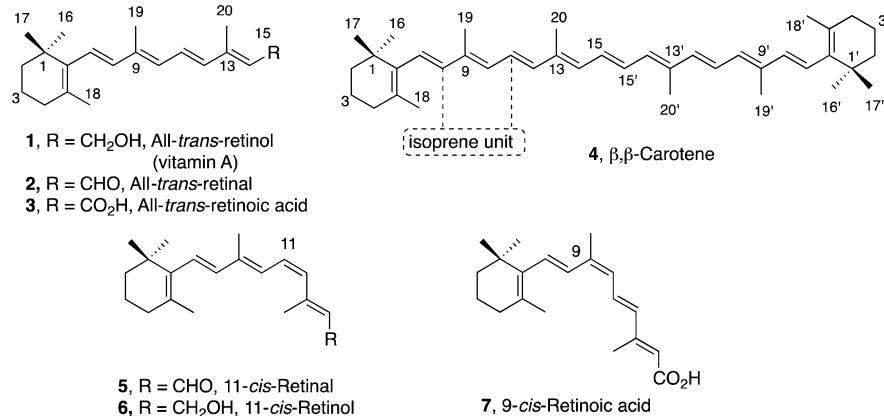
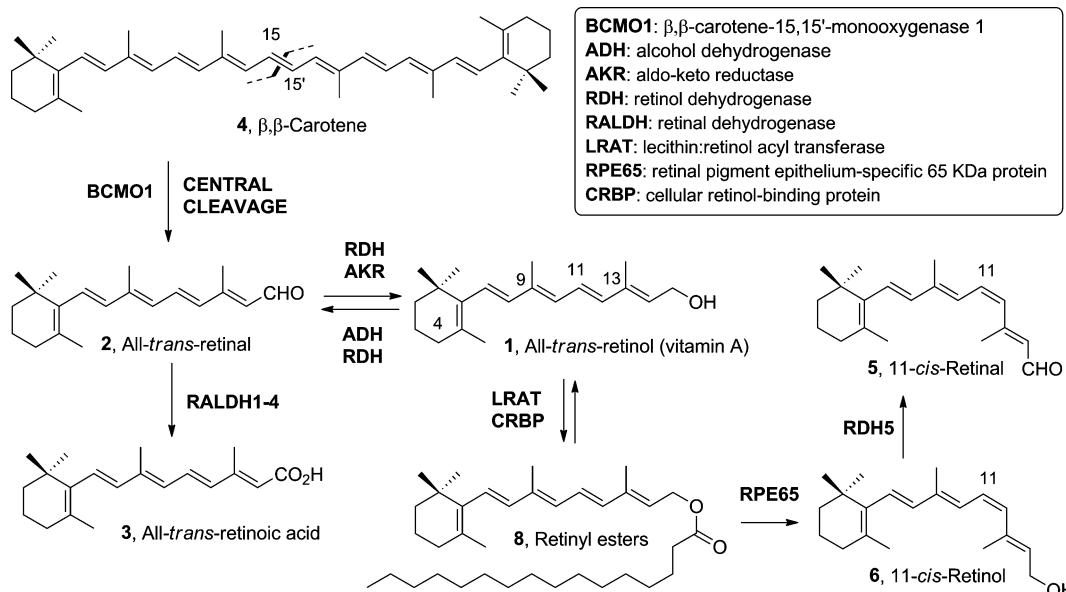


Figure 1. General structure and numbering of retinoids and carotenoids.⁴⁰

Scheme 1. Biogenesis of Retinoids from Pro-vitamin A Carotenoids Such as β,β-Carotene (4) and from Retinyl Esters^a



^aRetinyl palmitate 8 is shown.

Most of the biological processes linked to vitamin A are in fact due to the interaction of several metabolites with their cognate biological receptors. These metabolites are generated in vivo by redox changes affecting the functional group (retinal, retinoic acid), the allylic positions, and the conjugated polyene chain and/or by isomerization of some selected double bonds. Collectively, these compounds, which include vitamin A, its metabolites, and synthetic analogues that display (some of) the activities of the natural compounds, are called retinoids.^{2b,c,14}

George Wald determined in 1934 that 11-cis-retinal (**5**, Figure 1) and all-trans-retinal (**2**, Figure 1) are the chromophores of the visual pigments.¹⁵ Our understanding of the biochemistry and molecular biology of the visual cycle (see section 4.2) and the retinoid cycle (the conversion of all-trans-retinal via **1** and 11-cis-retinol **6** to 11-cis-retinal **5**, see Scheme 1) has increased enormously in the last years.¹⁶ Likewise, knowledge of the chemical composition of lipofuscin, which contains dimeric compounds made from vitamin A metabolites, and of the genetic defects in the enzymes that control the visual cycle and the retinoid cycle, are advancing our understanding of the etiology of eye diseases and suggesting therapeutic options.¹⁷

Other proteins used by microorganisms to control membrane ion homeostasis and phototaxis are based on light-induced photocycles driven by isomerization of the chromophore all-trans-retinal (**2**) bound to membrane proteins that are strikingly similar to the proteins of the visual cycle.¹⁸

Except in vision, most of the cellular processes under the influence of vitamin A and its analogues are mediated by the binding to (and activation of) two families of nuclear receptors¹⁹ as well as to retinoid metabolizing enzymes.²⁰ The structural and functional studies on nuclear receptors, and the identification of families of retinoic acid receptors, RARs [RAR α (NR1B1), RAR β (NR1B2), and RAR γ (NR1B3)],²¹ and retinoid X receptors, RXRs [RXR α (NR2B1), RXR β (NR2B2), and RXR γ (NR2B3)],²² that are activated by all-trans-retinoic acid (**3**) and its 9-cis isomer (**7**, Figure 1) have significantly deepened our understanding of the molecular mechanisms by which retinoids and ligands of the nuclear receptor superfamily in general confer the ability onto these inducible transcription factors to regulate target gene transcription²³ and set up dynamically diversifying gene networks.²⁴ Moreover, these studies have provided an exceptionally precise view on the structural features of a ligand and the allosteric

structural alterations induced in the cognate receptor that result in the various ligand activities, such as distinct types of agonistic or antagonistic activities.^{21–23,25}

The cancer preventive action of vitamin A was observed as early as 1925 by Wolbach and Howe.²⁶ In their classic paper describing the cellular effects of vitamin A deficiency in the rat, they noted effects on both differentiation and proliferation of epithelial cells. Indeed, during vitamin A deficiency, they found that proper differentiation of stem cells into mature epithelial cells failed to occur. In addition, they observed abnormal cell differentiation and noted excessive cellular proliferation in many of the deficient epithelia. On the basis of their results, they concluded that an adequate level of retinoids was necessary for control of normal cellular differentiation and proliferation. As pointed out by Sporn and Roberts nearly 50 years later,²⁷ these observations provided the rationale for the later attempts to exploit the (then unknown) retinoic acid signaling pathway for cancer therapy and cancer prevention. Dermatology and cancer have been the traditional therapeutic areas of retinoids, but the knowledge on RAR and RXR function as well as the understanding of the complex gene network modulated by the ligands is pointing to new therapeutic opportunities.²⁴ All-*trans*-retinoic acid/arsenic trioxide combination therapy (together with chemotherapy protocols primarily for postremission consolidation and maintenance therapy) of acute promyelocytic leukemia (APL) cures more than 90% of patients.²⁸ As far as RXR ligands (also called rexinoids) are concerned, the U.S. Food and Drug Administration (FDA) approved bexarotene in 1999 for the treatment of refractory cutaneous T-cell lymphoma (CTCL), and efforts are ongoing to dissociate activities that induce hypothyroidism and elevated triglyceride levels, presumably by affecting RXR heterodimer pathways for other nuclear receptors.²⁹ A limitation of all-*trans*-retinoic acid-based therapies is their teratogenicity.³⁰ Hypervitaminosis, the excess intake of vitamin A, may be harmful in the elderly due to adverse effects of vitamin A toxicity on bone loss.

Here, we provide the first in-depth review on retinoids and carotenoids, including not only their biogenesis but also their main roles in (patho)physiology, the therapeutic applications and novel promising paradigms for future therapies of some of these compounds, and the synthetic methodologies developed over the years. A PubMed search reveals about 100–200 yearly reviews on retinoids and carotenoids, highlighting that this is an active field of considerable interest to basic scientists, pharmacologists, and clinicians. The existing knowledge on retinoids has been comprehensively reviewed 20 years ago, and readers may consult the corresponding monographs for many of the basics concerning the biology, chemistry, and medicine of this class of compounds.^{2a–c,14a,c} Concerning carotenoids, there are excellent monographs that cover virtually all aspects of chemistry and biology: Vol. 1A: Isolation and Analysis,³¹ Vol. 1B: Spectroscopy,³² Vol. 2: Synthesis,³³ Vol. 3: Biosynthesis and Metabolism,³⁴ Vol. 4: Natural Functions,³⁵ and Vol. 5: Nutrition and Health.³⁶ Other monographs cover most aspects of carotenoid functions,¹³ in particular nutrition.^{2d} Recent review articles deal with the biogenesis and biological functions of carotenoids¹⁰ and their cleavage products, the so-called apocarotenoids.^{10a}

Given the polyunsaturated skeletons of natural retinoids and carotenoids, we will cover herein the main synthetic approaches to polyenes, which will also be of interest to synthetic chemists. The Handbook of Carotenoids, edited by G. Britton, S. Liaaen-

Jensen, and H. Pfander in 2004,⁹ lists all known carotenoids as separate entries numbered according to the Key to Carotenoids published a few years before.³⁷ Entries for the well-characterized compounds provide structures, spectroscopic data, and additional information on sources and properties. References to synthesis are also provided. Rather than reproducing the excellent schemes provided in previous monographs,³³ we opted to provide systematic views of synthetic strategies as applied to both C₂-symmetric and nonsymmetric carotenoids, and at the same time discuss in detail the new generation of synthetic approaches that rely on contemporary developments in the construction of polyenes. We will not discuss the synthesis of retinoids designed for the modulation of the nuclear receptors, which contain generally (hetero)aromatic rings in place of dienes/trienes of the parent compounds. This family of more than 4000 compounds has been extensively dealt with in recent review articles on their design, receptor-binding, and receptor modulation.^{14b,38} Similarly, general synthetic strategies for retinoids and carotenoids have also been covered previously.³⁹

2. STRUCTURE AND NOMENCLATURE OF RETINOIDs AND CAROTENOIDs

Retinoids are formally diterpenes (C₂₀) because they are composed of four isoprene (C₅) units joined in a head-to-tail manner, but they are biosynthetically derived from the oxidative cleavage of carotenoids. Carotenoids and their oxygenated derivatives (xanthophylls) are tetraterpenes (C₄₀) composed of eight isoprene units joined in a head-to-tail fashion except the central unit, which has a reverse connection (Figure 1). Carotenoids and retinoids are undoubtedly the most important natural polyunsaturated isoprenoids.

Several prefixes, preceded by the number of the carbon atom(s) that contain the modification, are used for the systematic nomenclature of modified retinoids and carotenoids:⁴⁰ hydro or dehydro designates the addition or removal of hydrogens, nor applies to the elimination of CH₃, CH₂, or CH groups, retro describes isomerizations due to the shift of a hydrogen between two positions of the conjugated polyene, apo designates the shortening of the skeleton by the formal removal of fragments from one or both ends, seco describes the fission of a bond between adjacent carbons, and epoxy is used for designation of oxygen bridges.

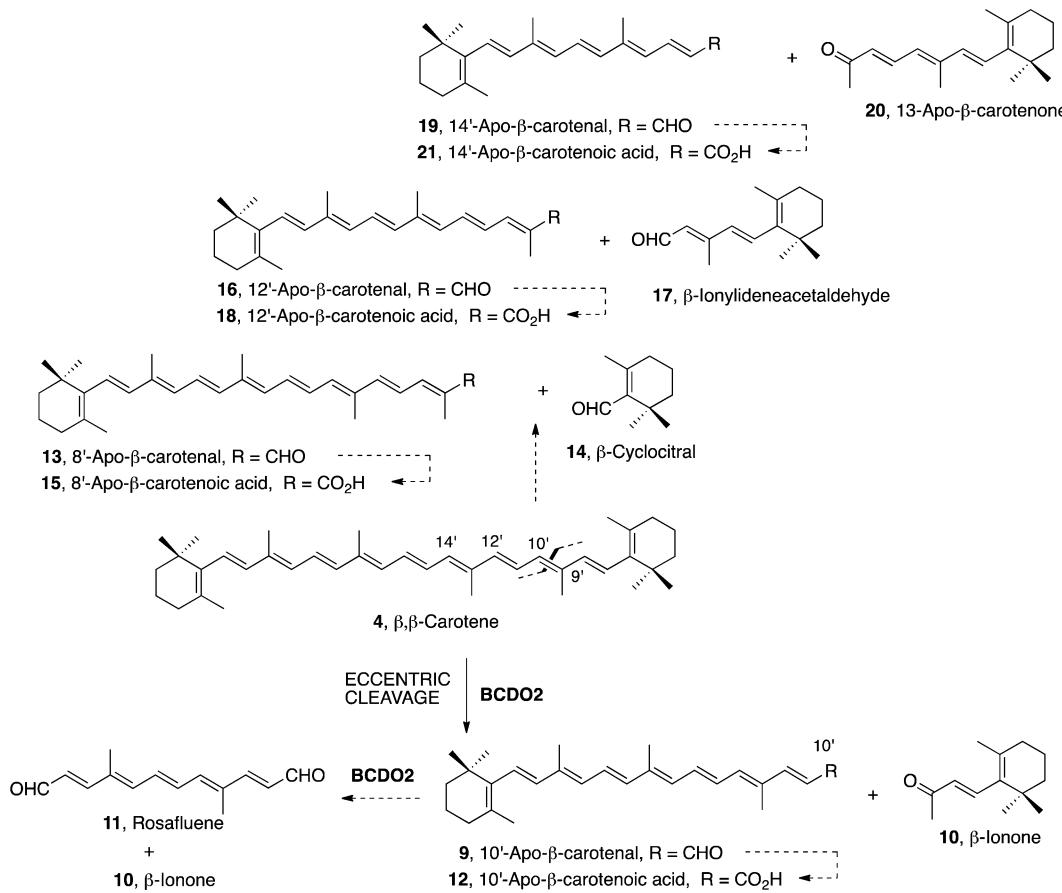
Specific names for end-group designation of carotenoids are explained in section 9.

3. BIOGENESIS OF RETINOIDs, ABSORPTION, AND TRANSPORT

3.1. Pro-vitamin A Carotenoids and Apocarotenoids

In 1930, the first evidence of the dietary relationship between a plant-derived carotenoid (β,β -carotene **4**) and vitamin A (**1**) was established from studies on rat small intestine.⁴¹ Karrer et al. elucidated the structure of β,β -carotene (**4**) and proposed a central cleavage of this carotenoid for the formation of all-*trans*-retinal (**2**).⁴² At present, two pathways, termed central and eccentric, are known to operate in the enzymatic conversion of β,β -carotene (**4**) to retinoids. The enzymes responsible for the cleavage are β,β -carotene-15,15'-monooxygenase 1 (BCMO1) and β,β -carotene-9',10'-dioxygenase 2 (BCDO2).

BCMO1 is a member of the carotenoid cleavage enzyme (CCE) family of nonheme iron oxygenases that has been characterized in plants, mammals, fungi, and bacteria.⁴³ It

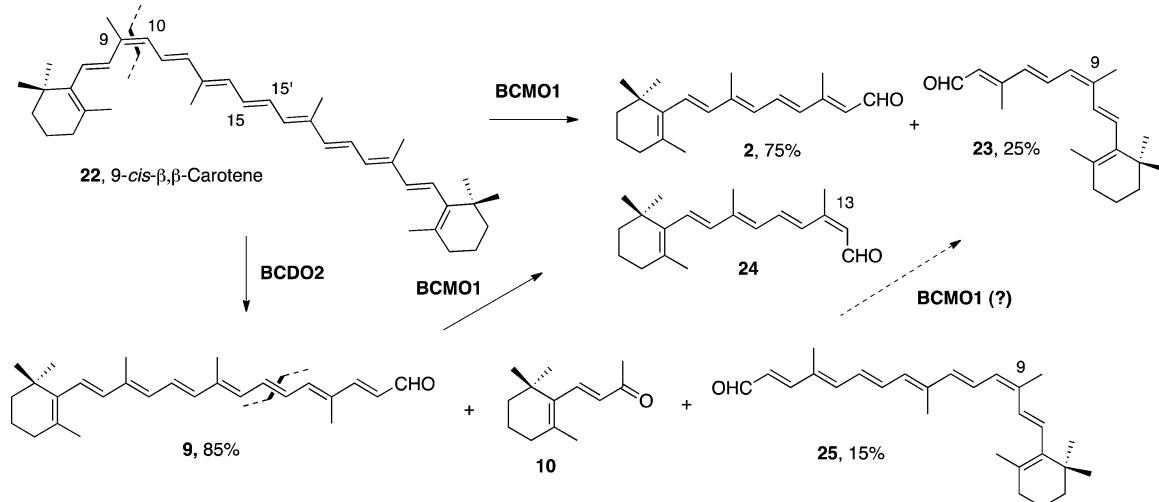
Scheme 2. Biogenesis of Apo- β -carotenoids from β,β -Carotene (4) by the Action of BCDO2^a

^aDashed lines indicate putative biogenetic connections.

catalyzes the central cleavage at the C15=C15' bond of β,β -carotene (4) to produce two molecules of all-trans-retinal (2) (Scheme 1).^{43,44} The mechanistic details of the bond excision process are, however, still unclear. Under aerobic conditions, the cleavage reaction has been proposed to occur either by formation and decomposition of an unstable dioxetane intermediate (dioxygenase reaction),⁴⁵ or by formation of an epoxide and its opening by water prior to the cleavage step (monooxygenase reaction).^{45a} Nonetheless, an alternative mechanism supported by mutational studies and molecular modeling has been suggested that involves the formation of a carbocation intermediate stabilized by aromatic residues in the substrate binding pocket.⁴⁶ BCMO1 is a cytoplasmic protein that requires an intact ionone ring, and therefore is specific for pro-vitamin A carotenoids, such as β,β -carotene, α -carotene, and β -cryptoxanthin. Cellular retinol-binding protein type I (CRBP-I) acts as intracellular sensor of vitamin A status, and in the unbound (apo) form it stimulates BCMO1 activity to produce retinoids.⁴⁷ Prior to the rapid conversion into all-trans-retinal (2) in enterocytes by the action of BCMO1, carotenoids must be absorbed in the small intestine. This process is facilitated by scavenger receptors SR-B1 (scavenger receptor class B type 1) and CD36 (thrombospondin receptor).⁴⁸ The β,β -carotene that is not cleaved in the intestine (about 40%) and stored in circulating chylomicrometers is metabolized in peripheral tissues. BCMO1 is expressed in developing tissues,⁴⁹ and its absence exacerbates vitamin A deficiency in models lacking retinol-binding proteins (RBPs).

Moreover, BCMO1 influences vitamin A stores independently of carotenoid metabolism by a yet unknown mechanism,^{49,50} and also regulates fat body reserves.⁸ In vivo studies have revealed that long-term supplementation with β,β -carotene (4) reduces adiposity in mice, an effect mediated by the reduction of the activity in adipocytes of the transcription factor peroxisome proliferator activated receptor γ (PPAR γ , NR1C3), a member of the nuclear receptor (NR) superfamily, which is itself due to BCMO1-dependent cleavage of β,β -carotene (4) to retinoids.⁵¹

BCDO2 is a mitochondrial enzyme that forms apo-carotenoids by cleavage at the C9=C10 and C9'=C10' bonds, forming 10'-apo- β -carotenal (9) and β -ionone (10) from β,β -carotene (4) (Scheme 2).⁵² BCDO2 shows broader substrate specificity than BCMO1,⁵³ and accepts not only carotenoids with intact β -ionone rings as substrates but also xanthophylls, ϵ -carotene, and even the acyclic lycopene. In addition, 10'-apo- β -carotenal (9) serves as substrate for a further oxidative cleavage, providing rosafluene (11) and another molecule of β -ionone (10).^{8,52b} Although the eccentric cleavage could potentially yield a series of apo- β -carotenals/apocarotenones (13, 16, and 19, and their complementary fragments 14, 17, and 20, respectively) depending upon the position of the bond cleavage (Scheme 2), only the products of cleavage at the C9'=C10' bond (9 and derivatives, and β -ionone 10) have been characterized in mice, and therefore the occurrence of enzymatic excision reactions at other positions is unclear.⁸ In any case, apo- β -carotenoids can also be formed

Scheme 3. Enymatic Cleavage of Dietary 9-cis- β,β -Carotene (22)

from β,β -carotene (4) by nonenzymatic autoxidation processes. Because of their structural similarity to retinoids, some of these apocarotenoids have been shown to function as signaling molecules (vide infra).⁵⁴

The eccentric cleavage, a minor pathway relative to the central cleavage, could however occur preferentially under certain oxidative conditions (smoking, oxidative stress, etc.) or under conditions of excess β,β -carotene (4).⁵⁵ Studies in animal models have shown that carotenoids accumulate in the mitochondria, where BCDO2 is localized, and impair respiration, causing oxidative stress and inducing signaling pathways related to cell survival and proliferation. This might explain some adverse health effects of carotenoids and identify BCDO2 as an important target for their prevention.⁸

Dietary 9-cis- β,β -carotene (22) is processed by both BCMO1 and BCDO2 to provide a mixture of all-trans-retinal (4), 9-cis-retinal (23), and 13-cis-retinal (24) in a 9:3:1 molar ratio (Scheme 3).⁵⁶ Thus, BCMO1 also has isomerase activity, and the partial isomerization must occur in the central cleavage step to yield the ca. 3:1 ratio of E/Z isomers observed. BCMO1 shows a higher affinity for 9-cis- than for β,β -carotene, and the preferential interaction with the 9-cis moiety of 9-cis- β,β -carotene (22), together with the possible formation of a carbocation intermediate⁴⁶ that favors isomerization, could explain the discrepancy with respect to the theoretically expected 1:1 ratio. BCDO2 appears to remove the 9-cis site, and the all-trans-10'-apo- β -carotenal (9) (perhaps, also the 9-cis isomer 25) must undergo further cleavage by BCMO1.⁵⁶ Animal studies indicate that dietary 9-cis- β,β -carotene (22) is not a major source of 9-cis-retinoids, but instead supplies the retinoids of all-trans configuration required to sustain vitamin A action in the mouse.⁵⁶

3.2. Metabolism of Retinoids at the Functional Group

The reduction of all-trans-retinal (2) is catalyzed by NAD-dependent dehydrogenases (retinol dehydrogenases, RDHs) of the cytosolic medium-chain (MDR) and the membrane-bound short-chain (SDR) dehydrogenases/reductases,^{20b,57} and by aldo-keto reductases (AKR).⁵⁸ Dietary retinol is absorbed in the small intestine, and up to 90% of total body retinol can be stored in the liver as retinyl esters of long chain fatty acids (such as retinyl palmitate 8, Scheme 1) through the action of lecithin:retinol acyl transferase (LRAT).⁵⁹ Three cellular retinol

binding proteins (CRBP, Figure 2)⁶⁰ facilitate the esterification of retinol by LRAT, and probably protect free all-trans-retinol

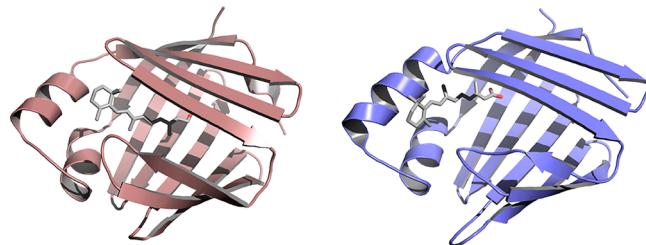


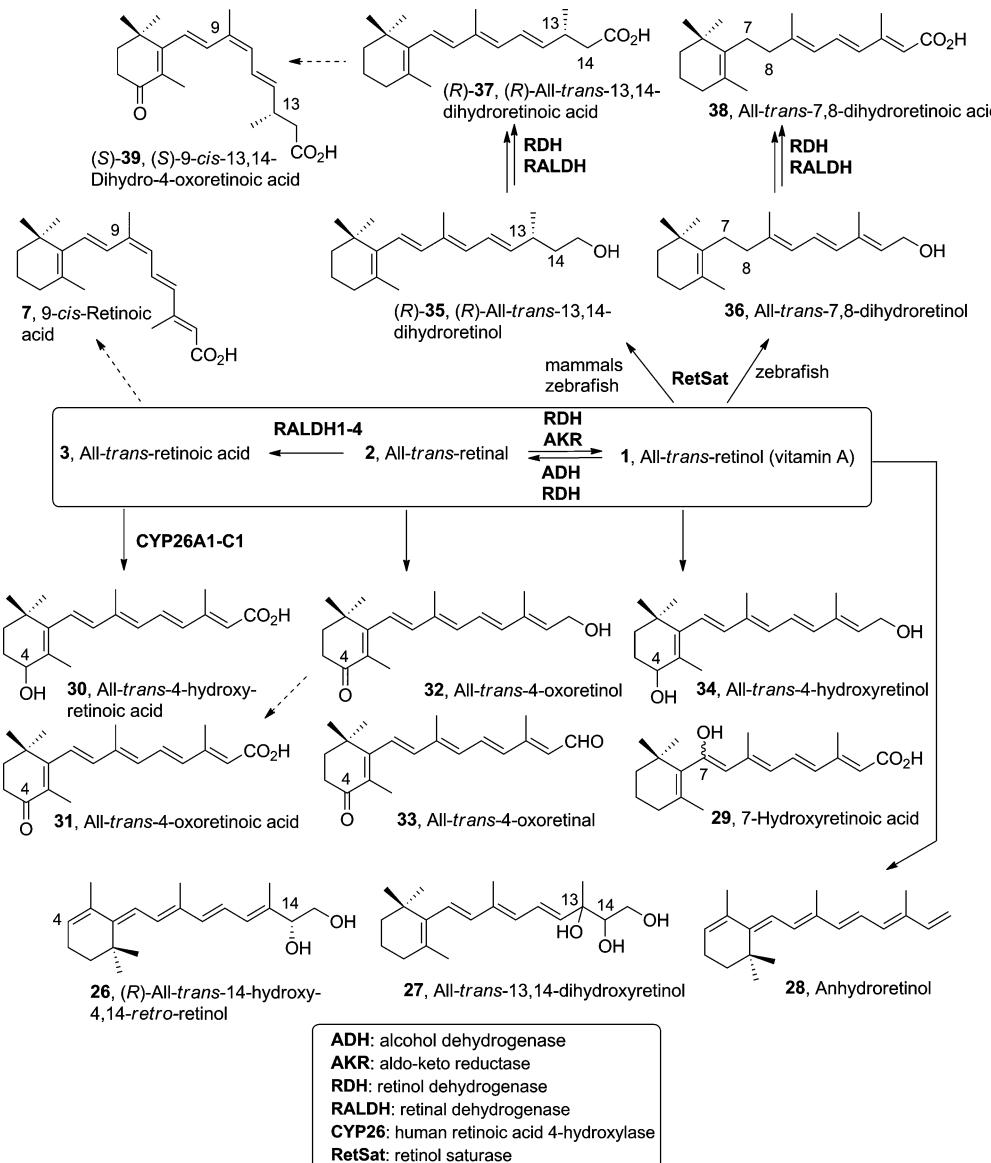
Figure 2. Representation of the three-dimensional structures of CRBP-I (PDB: 1crb) (left)⁶¹ and CRABP-I (PDB: 1cbr)⁶³ members of the intracellular lipid-binding protein family.

(1) from oxidative processes.⁴⁸ The CRBP protein is composed of 10 antiparallel β -sheet that folds back into itself and forms thereby an orthogonal barrel that constitutes the ligand binding pocket (LBP).⁶¹ The ligand adopts an extended 6-s-trans conformation with the β -ionone ring located in a hydrophobic niche, whereas the alcohol forms a hydrogen bond to Glu₁₀₈. A charged amine residue from Lys₄₀ stays close to the terminal isoprene unit.⁶¹

CCEs in animals can additionally isomerize the C11=C12 bond of the polyene, which is important for the formation of the 11-cis isomers required for vision.^{8,62} Whereas in insects a single enzyme (NinaB, which acts following β,β -carotene transport to the adult brain after cellular uptake by the membrane “scavenger” receptor NinaD) carries out the two functions, in mammals the combined action of BCMO1 and RPE65 (retinal pigment epithelium 65 kDa protein) is needed for cleavage and isomerization, respectively. RPE65 is the retinoid isomerase of the visual cycle and catalyzes the conversion of retinyl esters (8) to 11-cis-retinol (6, Scheme 1).⁶²

Mobilization of retinol to extrahepatic tissues requires the secretion of serum retinol binding protein (RBP4), which is the main carrier in blood.⁶⁴ Transport of retinol in the blood is controlled by sRBP (lipocalin serum retinol binding protein), which is synthesized in the liver and released as a complex with transthyretin (transporter of thyroxin and retinol, TTR). In the blood, free sRBP interacts with a sRBP receptor (RBPR) in the

Scheme 4. Biogenetic Routes of Other Naturally Occurring Retinoids Modified at the Hydrophobic Ring and/or the Side-Chain



plasma membrane of cells, which is followed by the release of retinol and its uptake into the cells.⁶⁵ The newly discovered RBP4-receptor 2 (RBPR2) is another retinol transporter expressed in liver and intestine.⁶⁶ Stimulated by retinoic acid 6 (Stra6) is a high-affinity receptor expressed in several tissues (not in liver or intestine), which mediates retinol transfer from RBP4 to cells.⁶⁵

Alternatively, oxidation of all-trans-retinal (2) to all-trans-retinoic acid (3) (Scheme 1) is carried out by aldehyde dehydrogenases (ALDH or RALDH), which are also cytosolic proteins.^{20b,57} After being synthesized, all-trans-retinoic acid (3) is transported in a complex with cellular retinoic acid binding proteins (CRABP-I and -II)⁶⁷ and delivered to the cell where it binds to nuclear receptors (retinoic acid receptors, RARs) to induce biological responses. The X-ray structure of CRABP-I⁶³ (Figure 2) is similar to that of CRBP-I, with two orthogonal, five stranded β -sheets and a helix-turn-helix motif located at the entrance of the ligand-binding pocket. The β -ionone ring is close to the surface of the protein, the contacts are predominantly hydrophobic, and the carboxylic acid interacts

with a trio of residues (Arg₁₃₂, Tyr₁₃₄, and Arg₁₁₁; CRABP-II numbering).

Although it binds to and activates both RARs and RXRs, 9-cis-retinoic acid (7) is unlikely a bona fide physiological ligand, because it has not been detected in culture or in vivo (an exception may be the pancreas in adult mouse)⁶⁸ unless all-trans-retinoic acid (3) is present. Pharmacological and genetic studies in mouse epidermal keratinocytes⁶⁹ and mouse embryos also failed to confirm such a function.^{20a,70} Enzymes that oxidize exogenous 9-cis-retinol to 9-cis-retinal have been characterized.⁷¹ It should be pointed out that experiments addressing this issue are demanding, as UV photoisomerization (from regular laboratory illumination) or thiol-induced isomerization of all-trans-retinoic acid (3) produces a mixture of isomers containing 8–9% 9-cis-retinoic acid (7). Liver microsomes likewise can nonenzymatically catalyze this isomerization.⁷²

Retinoid metabolism is therefore a complex and well-orchestrated process that depends on the relative contribution of each enzyme class, the kinetic constants and affinities, and

partitioning of retinoid pools between membranes and cytoplasm.^{20b,48,57} For example, genetic and animal studies suggest that all-*trans*-retinoic acid (3) produced from dietary precursors regulates, via the induction of the gut homeodomain transcription factor ISX, the intestinal expression of SR-B1 and BCMO1,⁸ perhaps accounting for the observation that β,β -carotene supplementation does not induce hypervitaminosis A.

3.3. Metabolism of Retinoids at the Side-Chain and at the Hydrophobic Ring

Vitamin A acts as a prohormone in cells and organs, generating a diversity of metabolites that play key roles in fundamental physiological processes. In addition to the analogues generated by changes in the polar group oxidation level, other intracellular mediators derive from all-*trans*-retinol (1) by biosynthetic pathways that induce structural changes to the cyclohexenyl ring and/or to the side-chain (double-bond geometry, positional shifts involving allylic hydrogens and side-chain double-bond dehydro- and hydrogenation).

3.3.1. Oxidation of Retinol at the Side-Chain. 14-Hydroxy-4,14-*retro*-retinol (14-HRR, 26) and 13,14-dihydroxyretinol (13,14-DHR, 27) are vitamin A metabolites with additional hydroxyl groups on the side-chain (Scheme 4). These compounds act as immunological messengers, inducing B-cell proliferation and T-cell activation. 14-HRR (26), isolated from the lymphoblastoid 5/2 cell line and from HeLa cell cultures, was the first bioactive *retro*-retinoid described.⁷³ 14-HRR (26) sustains the growth of B lymphocytes and the activation of T lymphocytes at 20–30-fold lower concentrations than all-*trans*-retinol (1).⁷⁴ The compound isolated from cells in minute amounts was a mixture of enantiomers with the R enantiomer exhibiting slightly higher activity than the S.⁷⁵ 13,14-DHR (27) was identified in lymphoblastoid 5/2 cells grown in the presence of all-*trans*-retinol (1) and shown to support the viability of retinol-deprived lymphocytes, which in these conditions show reduced potential for proliferation of their B cells and nearly complete block of cell activation.⁷⁶ Although 14-HRR (26) can be obtained from 13,14-DHR (27) by mild acid treatment, cellular studies have shown this not to be the case *in vivo*, and therefore both retinoids are independent end-products of retinol metabolism.⁷³ Whereas 14-HRR (26) and 13,14-DHR (27) act as growth-promoting factors for retinol-dependent cells, another retinol metabolite, anhydroretinol (AR, 28), showed growth-inhibitory activity. The *retro*-retinoid AR (28) (Scheme 4) was first isolated from fish liver oils and later from human B cells and *Drosophila* Schneider S2M3 cells, but was also detected in several other cell lines from both Chordata and Arthropoda phyla.⁷⁷ AR (28), which might be biogenetically obtained from all-*trans*-retinol (1) through the action of a dehydratase,⁷⁸ functions as an antagonist of both all-*trans*-retinol (1) and 14-HRR (26) actions. It blocks B lymphocyte proliferation and the activation of resting T lymphocytes. 14-HRR (26) and AR (28) are the first agonist/antagonist pair of lipid-signaling molecules reported. AR (28) also induces oxidative stress and cell death,⁷⁹ as confirmed by the production of singlet oxygen and superoxide upon UV irradiation.⁸⁰ F-actin was identified as a functional target for *retro*-retinoids, because F-actin reorganization of several lymphocyte and fibroblast cell lines was noted as an early event in AR-triggered apoptosis.⁸¹

7-Hydroxyretinoic acid (29, Scheme 4) has recently been isolated from cell cultures of some cyanobacteria (*Microcystis aeruginosa* and *Spirulina* sp.)⁸² and shown to exist as a mixture

of four isomers due to enol–keto tautomerism and *cis*–*trans* isomerism. The *in vitro* RAR agonistic activity of this metabolite is about 50% relative to all-*trans*-retinoic acid (3).

3.3.2. Oxidation of Retinoids at the Hydrophobic Ring. Further catabolism of all-*trans*-retinoic acid (3) to more oxidized metabolites occurs primarily through the action of the cytochrome P450 family of hemoproteins, which finally results in retinoid inactivation. All-*trans*-retinoic acid (3) is metabolized mainly by CYP26A1,⁸³ but other P450 enzymes such as CYP2C8 and CYP3A also contribute to hydroxylation at position C4. The major metabolic products of all-*trans*-retinoic acid are all-*trans*-4-hydroxyretinoic acid (30) and all-*trans*-4-oxoretinoic acid (31) (Scheme 4). The (S)-all-*trans*-4-hydroxyretinoic acid (S)-30 is preferred over the (R) enantiomer by the enzymes regulating all-*trans*-retinoic acid homeostasis, and is the exclusive enantiomer produced by CYP26A. However, all-*trans*-4-oxoretinoic acid (31) was formed by CYP26A1 from (R)-all-*trans*-4-hydroxyretinoic acid but not from the (S) enantiomer (S)-30.⁸⁴ Thus, enantiodiscrimination at the level of these oxidative enzymes establishes a delicate control of the homeostasis of retinoids.⁸⁵ In addition, metabolites corresponding to the oxidation at the C18 allylic ring methyl substituent (18-hydroxy-retinoic acid) have been identified.⁸⁶

Metabolites of vitamin A obtained by oxidation at the C4 position are also bioactive agents rather than inactive catabolites.⁸⁷ All-*trans*-4-oxoretinol (32) induces differentiation of F9 cells and causes axial truncation in *Xenopus* embryos at the blastula stage, and these effects are not due to its conversion to all-*trans*-4-oxoretinoic acid (31). All-*trans*-4-oxoretinol (33, Scheme 4), the major retinoid metabolite isolated from *Xenopus* embryos,⁸⁸ binds to and transactivates through the RARs, and serves as a metabolic precursor of both all-*trans*-4-oxoretinol (32) and all-*trans*-4-oxoretinoic acid (31)⁸⁶ (Scheme 4). All-*trans*-4-oxoretinol (32), which is present in differentiated murine embryonic F9 stem cells (and other teratocarcinoma and embryonic stem cells) as a retinol metabolite more potent than putative precursor all-*trans*-4-hydroxyretinol (34), is considered as a novel signaling retinoid because it activates RAR, although it lacks the terminal carboxylic acid, and does not bind to or transactivate RXR.⁸⁹ In mouse skin, the topical treatment with all-*trans*-4-oxoretinol (33) or all-*trans*-4-oxoretinol (32) exerts a moderate direct retinoid-like activity (significant epidermal hyperplasia and metaplasia) similar to that induced by all-*trans*-retinol (1) and all-*trans*-retinal (2). These effects appear to be independent of RAR binding.⁸⁷

3.3.3. Reduction of Retinoids at the Side-Chain. Endogenous dihydroretinoids⁹⁰ are biosynthesized from vitamin A through a net hydrogenation reaction mediated by the enzyme retinol saturase, RetSat.⁹¹ RetSat enantiospecifically saturates all-*trans*-retinol (1) to produce (R)-all-*trans*-13,14-dihydroretinol (R)-35 (Scheme 4).^{90a} A related enzyme in zebrafish catalyzes the saturation of the C7=C8 bond in addition to the C13=C14 bond of all-*trans*-retinol (1) to produce both (R)-35 and all-*trans*-7,8-dihydroretinol (36). These metabolites undergo oxidation by SDR/MDR dehydrogenases, leading to the formation of (R)-all-*trans*-13,14-dihydroretinoic acid ((R)-37) and all-*trans*-7,8-dihydroretinoic acid (38) (Scheme 4). Both compounds are highly selective agonists that activate RAR but not RXR.^{90a,b} Similarly to all-*trans*-retinoic acid (3), levels of (R)-37 are controlled *in vivo* in both a temporal and a spatial manner through enzymes and transport factors involved in their synthesis and breakdown.⁹²

RetSat plays an important role in the biology of adipocytes, because it is induced during adipogenesis and is directly regulated by PPAR γ .⁹³ *RetSat*^{-/-} mice, deficient in (*R*)-all-*trans*-13,14-dihydroretinol ((*R*)-35), exhibit increased adiposity and increased expression of adipogenic markers while maintained on a high-fat diet. These results suggest that RetSat-deficiency is associated with alterations in the adipogenic program in vivo leading to increased fat deposition.⁹⁴ RetSat is thus a novel target for therapeutic intervention in metabolic diseases.⁹³ In addition, all-*trans*-retinal (2) may also affect adipogenesis, as in ob/ob mice it has been reported that administrating 2 or inhibiting the catabolizing enzyme RDH1 reduced fat and increased insulin sensitivity.⁹⁵

(*S*)-9-*cis*-13,14-Dihydro-4-oxoretinoic acid ((*S*)-39) (Scheme 4) is an endogenous retinoid metabolite with 9-*cis* configuration isolated from the liver (and present in other tissues) of mice, rats, and humans. It binds at least two RAR subtypes (α and β) and induces morphological changes in chicken limb buds similarly to all-*trans*-retinoic acid (3). This compound regulates gene transcription in the same organ although with lower potency than all-*trans*-retinoic acid (3).⁹⁶

3.4. Formation of Derivatives at the Functional Group

Retinoyl β -glucuronide was identified as a major metabolite in rat bile⁹⁷ and later shown to be biosynthesized in the small intestine from precursor all-*trans*-retinoic acid (3) as a mixture of all-*trans* and 13-*cis* isomers that was secreted in the bile.⁹⁸ It is also present as an endogenous compound in blood.⁹⁹ This carbohydrate derivative showed cellular activities similar to those of precursor 3, as measured by the induction of differentiation of acute promyelocytic leukemia cell lines and by the promotion of growth of vitamin A deficient rats, although it does not bind to the CRABP proteins or the RAR receptors. In addition, preclinical studies have shown that this metabolite is less toxic than all-*trans*-retinoic acid (3).¹⁰⁰

Retinoyl serine and retinoyl alanine are natural peptides produced by the moth *Trichoplusia ni*, but the configuration of the amino acids could not be established from the minuscule amounts isolated.¹⁰¹

4. RETINAL-BASED PROTEINS

Retinal-based proteins are chromoproteins that function either as sensors or as ion pumps in several species across all domains, Archaea, Eubacteria, and Eukarya.^{18a} These light-sensitive proteins share a common fold of seven transmembrane (7TM) helices and bind a retinal chromophore through a protonated Schiff base (PSB) with a lysine residue located in helix seven (see Figure 3). The absorption maxima of each retinal-based protein are modulated by the ionic environment of the PSB in the binding pocket.^{18b}

The opsin genes are classified into two groups: Type I opsins are found in archaea, eubacteria, fungi, and algae, and Type II opsins are found in animals. Microbial type I opsins, which comprise more than 1000 members,¹⁰⁴ control proton gradients and maintain membrane potential and ionic homeostasis. This group includes the light-driven ion pumps bacteriorhodopsin (bR) and halorhodopsin (HR) and light-gated ion channels called channel rhodopsins (ChRs).^{18b} Other microorganisms use opsin-based photoreceptors, such as sensory rhodopsin (SR), to modulate flagellar movements in phototaxis.¹⁰⁵ In marine photic ocean zone, the light-activated ion pumps from proteobacteria called proteorhodopsins, PRs,¹⁰⁶ have been linked to the survival of bacterioplankton

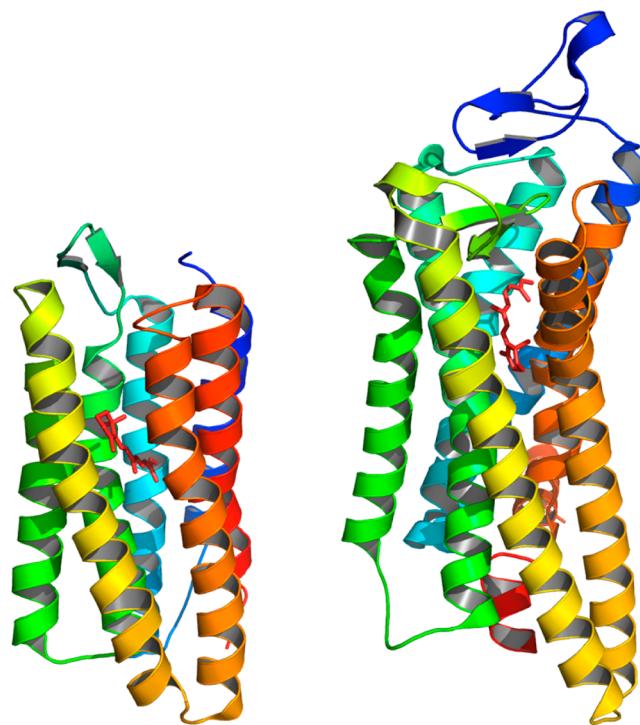


Figure 3. X-ray structures of bR (PDB: 1fbb) (left)¹⁰² and Rh (PDB: 1f88) (right).¹⁰³

during starvation periods.¹⁰⁷ Proton-pumping opsins have been found in eukaryotes (the fungus *Leptosphaeria maculans*).¹⁰⁸ Type II or animal opsins couple to G-protein coupled receptors (GPCR)-dependent signal transduction pathways that affect transmembrane ion currents. The best studied systems are the mammalian cone and rod visual pigments, but Type II opsins also control circadian rhythm and pigment regulation.^{18b}

In all opsin proteins, the absorption of a photon promotes the isomerization of the protein-bound retinal chromophore, all-*trans*- (2) to 13-*cis*-retinal (24) in Type I, and 11-*cis*- (5) to all-*trans*-retinal (2) in Type II opsins. This primary process triggers a series of structural changes leading to ion transport, channel opening, or interaction with signaling transducer proteins.

4.1. Microbial Type I Opsins. Rhodopsin Photoreceptors in Unicellular Organisms

All unicellular organisms use all-*trans*-retinal (2) bound to opsin in rhodopsin-like photoreceptors to capture energy and/or information from light sources and transform it into light-activated ion channels and pumps.^{18b,109} Light absorption induces isomerization of the chromophore from all-*trans*-retinal (2) to 13-*cis*-retinal (24, Scheme 3). In contrast to type II rhodopsin, the activated 13-*cis*-retinal chromophore in type I rhodopsins¹⁰⁴ remains covalently bound to its opsin protein partner and thermally reverts rapidly to the all-*trans*-retinal state without detaching from the protein. The efficiency of light absorption depends on the extinction coefficient of the complexes (ϵ_{max} typically between 50 000 and 70 000 M⁻¹ cm⁻¹) and the quantum efficiency (Φ , typically between 0.3 and 0.7). The turnover time of the photocycle for most light-driven pumps (HR and bR) is 10–20 ms.^{18b,109}

The structural features of the protonated Schiff base chromophore and the proton/ion conduction pathway regulate the absorption maxima of the pigments: ChR, $\lambda_{\text{max}} \approx 470$ nm;

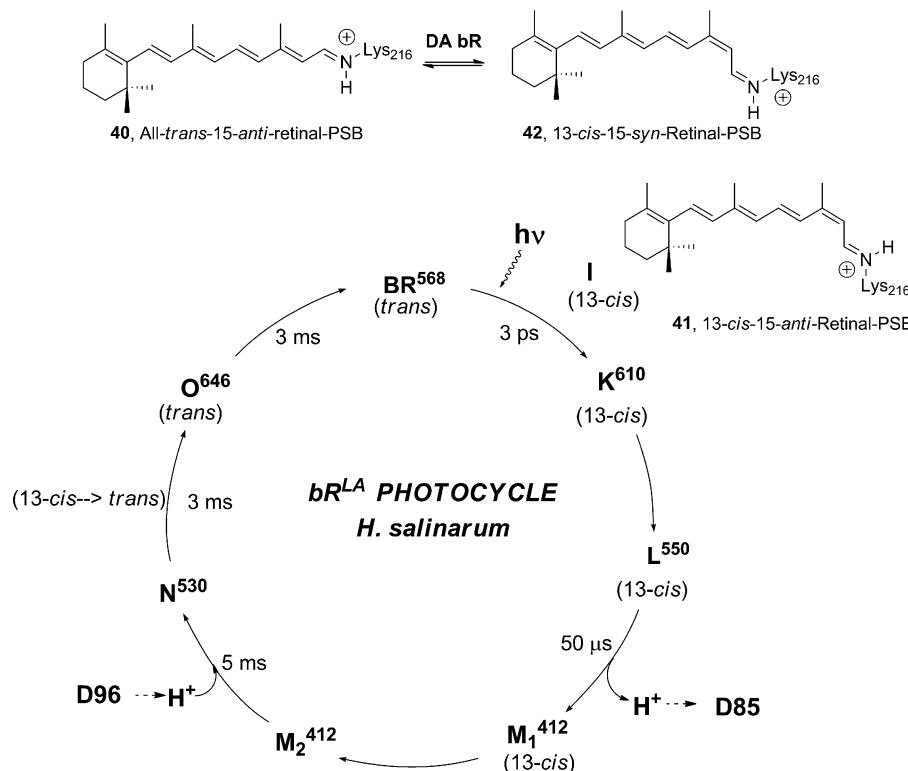


Figure 4. Light-adapted (LA) bacteriorhodopsin photocycle.

SRII, $\lambda_{\max} \approx 487$ nm; bR, $\lambda_{\max} \approx 568$ nm; HRs, $\lambda_{\max} \approx 580$ nm; SRI, $\lambda_{\max} \approx 587$ nm.¹¹⁰ The energy difference between ground (S_0) and excited (S_1) states of the rhodopsin-like proteins was initially considered to depend upon the planarity of the chromophore (a 6-*s-trans* conformation and an elongated, almost planar polyene chain for bR), the distance between the PSB and the counterion, and the interactions of the chromophore with amino acid residues in the binding pocket (the “two-point” charge model, which suggested the presence of a negative charge close to the hydrophobic ring).¹¹¹ The term “opsin shift”, defined as the difference between the protein absorption maximum and that of retinal-N-butylamine-PSB hydrochloride in MeOH, was coined to quantify the effect of the apoprotein on the absorption maximum of the retinal chromophore.¹¹²

4.1.1. Bacteriorhodopsin, a Light-Activated Proton Pump. The purple membrane (PM) of *Halobacterium salinarum* is a natural 2D crystal honeycomb lattice of bR trimers.¹¹³ The bR protein¹¹⁴ (for X-ray analysis, see refs 113,115 and Figure 3) converts light energy absorbed by the retinal chromophore covalently linked through a PSB to Lys₂₁₆ in helix 7 into a proton electrochemical gradient across the membrane. The absorption of light by the light-adapted bR form (which contains the all-*trans*-15-anti-PSB chromophore, 40) induces an ultrafast photocycle (complete in less than 30 ms), which starts with the isomerization of all-*trans*-retinal-PSB (40) to the 13-*cis* stereoisomer (with the 15-*anti* configuration, 41) on the “vibrationally hot” I state followed by a thermal relaxation process involving conformational changes of the retinal and the protein.¹¹⁶ Figure 4 depicts the photocycle of bR with the species spectroscopically characterized, the wavelength at which each intermediate maximally absorbs light and their lifetimes. Six discrete steps are recognized to account for the isomerizations (from BR⁵⁶⁸ to K⁶¹⁰ and from N⁵³⁰ to O⁶⁴⁶),

proton transport (from L⁵⁵⁰ to M₁⁴¹² and from M₂⁴¹² to N⁵³⁰), and accessibility changes (from M₁⁴¹² to M₂⁴¹² and from O⁶⁴⁶ to BR⁵⁶⁸) of the photocycle. A net transfer of one proton from the cytoplasm to the extracellular side of the membrane is produced under physiological conditions (pH > 7) as a result, and the ground-state configuration containing all-*trans*-retinal-PSB (40) is recovered.¹⁸ The proton transport sequence¹⁰³ comprises transfer of a proton to Asp₈₅, release of a proton from the proton release complex, reprotonation of the SB by Asp₉₆, uptake of a proton from the cytoplasm to reprotonate Asp₉₆, and the reprotonation of the proton release complex from Asp₈₅, followed by a final proton transfer from Asp₈₅ to Arg₈₂.^{113,117}

The dark-adapted bR consists of a mixture of all-*trans*-15-anti-PSB (40) and 13-*cis*-15-syn-PSB (42).¹¹⁸ The crystal structure of bR in the dark-adapted state with 13-*cis*-15-syn-retinal-PSB (42) revealed that the configuration changes due to retinal isomerization affect residues in the vicinity of the PSB, but most of the aromatic amino acids that surround the chromophore, and the polypeptide backbone of Lys₂₁₆, undergo small displacements.¹¹⁹

Two-photon absorption of bR leads to the loss of the crystalline order in the PM, although the bR trimers and the secondary structure are preserved. The structural changes induced by this photobleaching were accompanied by photo-reduction of the C15=N PSB bond, leading to N-retinylbacterioopsin photoproducts that show absorption maxima similar to the M state of bR.¹²⁰

The photochemistry and photophysics of bR have been the subject of intense investigations. Analogs of the native chromophore have yielded valuable structural, spectroscopic, and functional insights into the ground-state structure of the chromophore in the complex before X-ray structures became available,^{113,117} and continue to provide information on the

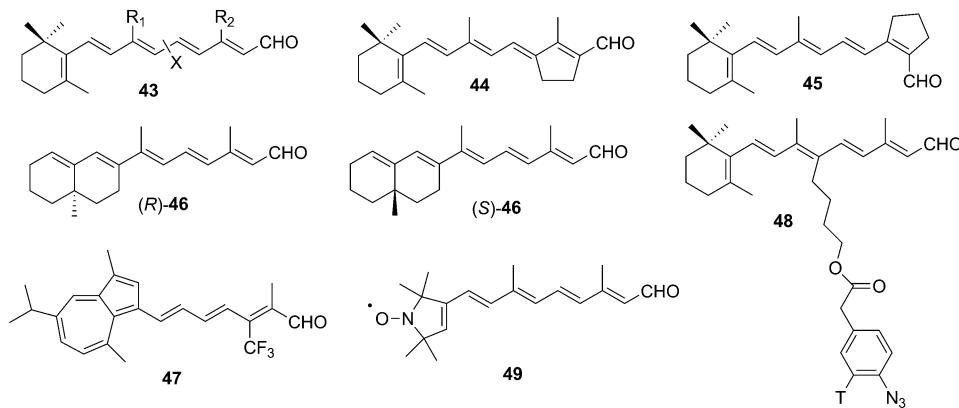


Figure 5. Selected retinal analogues for bioorganic studies of bR and other type I opsin.

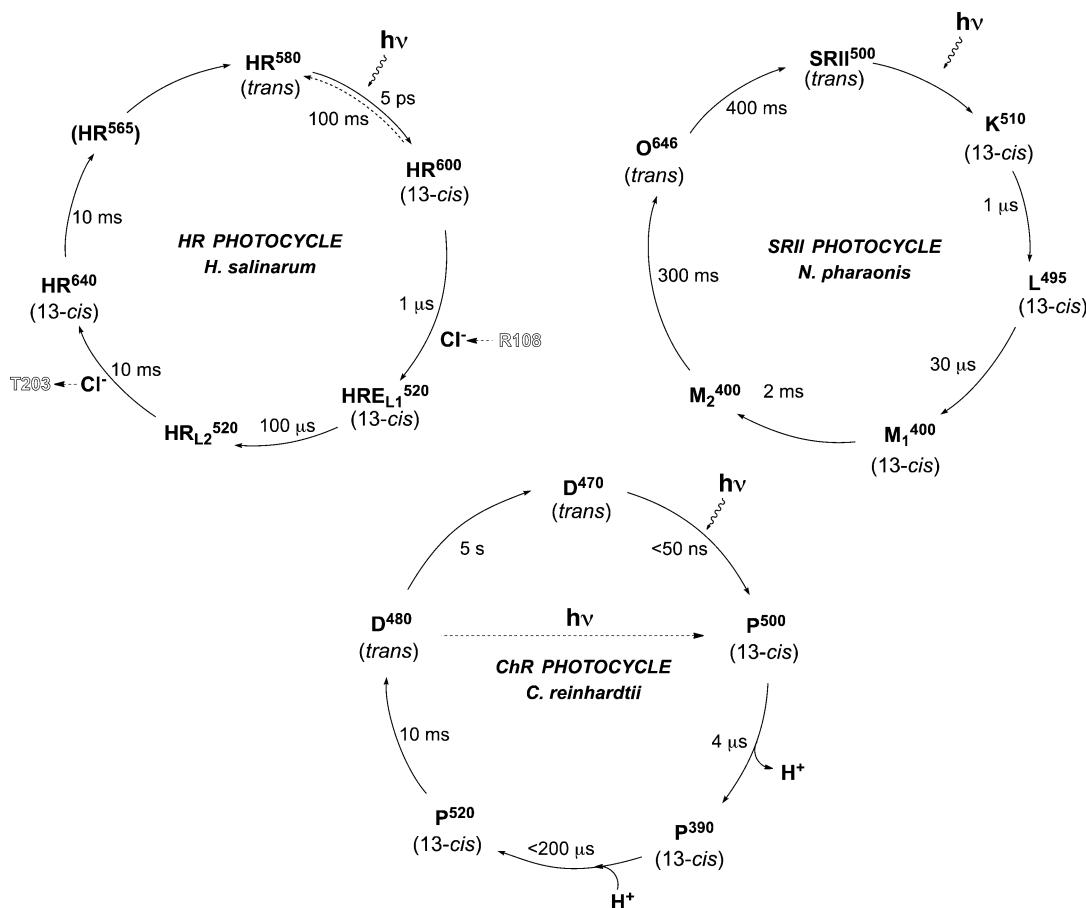


Figure 6. Photocycles of other bacterial opsin.

nature of the photocycle intermediates.¹²¹ Engineered by chemical synthesis, retinal analogues have been obtained with alterations on the polyene side-chain by substitution (demethylations, change of methyl positions, isotopomers and isotopologues, general structure 43, Figure 5), saturation of double bonds, incorporation of substituents (halogens, alkyl groups) and additional rings to lock conformations and/or configurations, and modifications on the cyclohexenyl ring. The ¹³C-labeled retinoids have been particularly useful for spectroscopic studies.¹²² A selection of these retinal analogues is shown in Figure 5. Locked retinals 44 and 45 provided chemical evidence that the primary photochemical step was the *cis/trans* isomerization of the C13=C14 bond.¹²³ Enantiopure

bicyclic derivatives 46 produced artificial pigments at different rate, and the higher yield of reconstitution of the *R* enantiomer indicated chiral recognition of bacterioopsin.¹²⁴ Guaiaculene analogue 47 provided an artificial bR with an absorption maximum at $\lambda_{\text{max}} \approx 830 \text{ nm}$.¹²⁵ The C10-substituted retinal with a tritiated phenylazide 48 was used to determine the orientation of the methyl groups within the protein binding pocket,¹²⁶ and the nitroxide retinal 49 allowed the use of electron spin resonance (ESR) to study this membrane protein. In the particular case of bR, the ESR signal before and after illumination confirmed that the chromophore ring is highly oriented and is kept rigid within the complex.¹²⁷

The ultrafast charge translocation that takes place on photoexcited retinal has been demonstrated by monitoring the absorption of tryptophans in the proximity of the chromophore in bR.¹²⁸ The absorption first decreases within 200 fs due to excitonic coupling between retinal and tryptophans and then recovers. Thus, a progressive change in dipole moment of the chromophore associated with changes in structure occurs during the time scale of the photoisomerization.¹²⁸ Optical control of the retinal isomerization in bR has been demonstrated using weak field conditions (where only 1 of 300 molecules absorb light), by which the amount of chromophore in the 13-*cis*-retinal configuration could be modulated by 20%.¹²⁹ Also, the population dynamics and the branching ratio between the all-*trans*- and 13-*cis*-retinal isomers can be modified by changes in pulse energy and duration.¹³⁰ As compared to the long, low intensity pulses, short intense pulses increase the isomerization yield by 50% due to the involvement of higher excited states (S_n).

Multipulse ultrafast transient absorption spectroscopy studies of all-*trans*-retinal-PSB led to the characterization of the S_1 reactive state and the determination of the photoisomerization rate.¹³¹ The branching ratio in the conical intersection (CI) between the S_1 and S_0 surfaces is considered the key factor that determines the quantum yield of isomerization of the PSB in solution, and this value was found to increase with solvent polarity.¹³¹ CASPT2//CASSCF/AMBER calculations of a protein model offered accurate information on the initial steps of the photocycle (bR to K), reproducing the experimental static and transient electronic spectra, the dipole moment changes, and the energy stored in the K photo-intermediate state. The opsin shift was ascribed to the conformational change of the chromophore from the 6-*s-cis* in solution to the fully planar 6-*s-trans* conformation in the complex and to the effect of the protein on dumping the counterion electrostatic effect relative to solution. Structural details of the bR photoisomerization process have also been proposed on the basis of these computations. The evolution of the excited-state intermediate I (known as the fluorescent state) toward a CI takes place with the deformation of the C10 to N moiety of the chromophore and the weakening/breaking of the N–H/water hydrogen bond network before formation of K.¹³² An asynchronous double “bicycle-pedal”¹³³ or “folding-table”¹³⁴ deformation, with the C11=C12 and C15=NH bonds twisting counterclockwise and the C13=C14 clockwise, is compatible with the calculations.¹³²

Systems based on the control of double-bond isomerizations are very useful for biological and technological applications.¹³⁵ By interfacing bR with solid supports (especially as a thin film), solid-state current-carrying electronic elements can be constructed for biomolecular optoelectronics.¹³⁶

4.1.2. Other Bacterial Opsins. The outward current-generating archaeal halorhodopsins (HRs) control gradients across the cell membrane by transporting chloride ions¹³⁷ from the extracellular medium into the intracellular space against an electrochemical potential to maintain osmotic pressure during cell growth.^{110,138} The primary photocycle (Figure 6),¹³⁹ although qualitatively similar to that of bR, does not show PSB deprotonation due to a single amino acid substitution of the Asp acceptor with Thr.¹⁴⁰ The X-ray structure¹⁴¹ shows a chloride ion in close proximity to the proton of the PSB.¹⁴¹ From HR⁶⁰⁰ to HRE⁵²⁰ a translocation of the chloride in the extracellular half-channel is produced loosening the Cl[−]–R₁₀₈ interaction and strengthening the Cl[−]–PSB bonding. The

chloride ion moves from the SB via Thr₂₀₃ to the cytoplasmic surface.

The photocycle of the behavioral photosensors SRI and SRII from *Natronobacterium pharaonis*¹⁴² (Figure 6) is similar to that of bR with analogous internal proton movements, but the light-initiated conformational changes of the opsin are used to activate a closely associated transducer molecule Htr.¹⁴³ When activated, Htr initiates a phosphorylation cascade that controls the directionality of the flagellar motor and directs phototaxis toward green and yellow light (SRI, $\lambda_{\text{max}} \approx 587$ nm) and away from blue light (SRII, $\lambda_{\text{max}} \approx 487$ nm). Uncomplexed SRII from *Natronomonas pharaonis* functions as a proton pump in the absence of Htr. Its crystal structure has provided insights into the switch of function of a bacterial opsin lacking the effector protein.¹⁴⁴ The X-ray structure of *Anabaena* SR after white-light illumination reveals the presence of a mixture of all-*trans*,15-*anti*- and 13-*cis*,15-*syn*-retinal-PSB chromophores, similarly to dark-adapted bR (see Figure 4). Because the chromophore ratio depends on the wavelength of irradiation, *Anabaena* SR constitutes a good model for the study of natural chromatic adaptation.¹⁴⁵

Channel rhodopsins (ChRs)¹⁴⁶ are used by microalgae to passively conduct nonselective (Na⁺, K⁺, and even Ca²⁺ ions) cations across the cellular membrane. Channel rhodopsins-1 and -2 (ChR1 and ChR2) were identified as light-gated ion channels in *Chlamydomonas reinhardtii*, a green unicellular alga from temperate freshwater environments.¹⁴⁷ The photocycle of ChR is similar to that of bR with the expected spectral differences (Figure 6),¹⁴⁸ due to variations in the ionic environment near the retinal Schiff base^{146b} and to differences in conformational changes within the protein.^{146b,149} The D⁴⁷⁰ dark state is converted by a light-induced isomerization of the all-*trans*-retinal-PSB chromophore via the early intermediate P⁵⁰⁰ and the transient P³⁹⁰ (which contains the deprotonated Schiff base in a state where the protein has undergone small conformational changes) to the P⁵²⁰ conducting state.¹⁴⁹ The recovery of the D⁴⁷⁰ dark state proceeds either thermally via the nonconducting D⁴⁸⁰ intermediate or photochemically via possible short-lived intermediates.^{148a} The desensitized D⁴⁸⁰ state can also be activated to yield the early intermediate P⁵⁰⁰. Other parallel cycles may be present.^{148,150}

A rhodopsin isolated from *Chlamydomonas* eyespot has been characterized as a family member of the histidine kinase rhodopsins (HKRs), which consist of Rh, a histidine kinase, a response regulator, and in some cases an effector. The chromophore in the recombinant Rh–UV state ($\lambda_{\text{max}} \approx 380$ nm) is a deprotonated 13-*cis*,15-*anti*-retinal-SB that photoconverts into Rh-Bl ($\lambda_{\text{max}} \approx 490$ nm), containing an equilibrium mixture of 13-*cis*,15-*syn*- and all-*trans*,15-*anti*-retinal-SBs that thermally equilibrate by double isomerization. The photochromic HKR is thought to be important for the behavioral adaptation of *Chlamydomonas* to UVA light.¹⁵¹

Middle rhodopsin (MR), recently isolated together with a bR-related rhodopsin from the bacteria *Haloquadratum walsbyi*, is the first microbial rhodopsin known to have 11-*cis*-retinal (S) as chromophore (similar to Type II rhodopsins) in addition to all-*trans*- and 13-*cis*-retinal. MR is evolutionarily transitional between bR and SRII. It shows an absorption maximum at $\lambda_{\text{max}} \approx 485$ nm, similarly to SRII, and a bR-like photocycle that however lacks proton-pumping activity. Therefore, this Rh may be a missing link in the evolution from Type I to Type II rhodopsins.¹⁵²

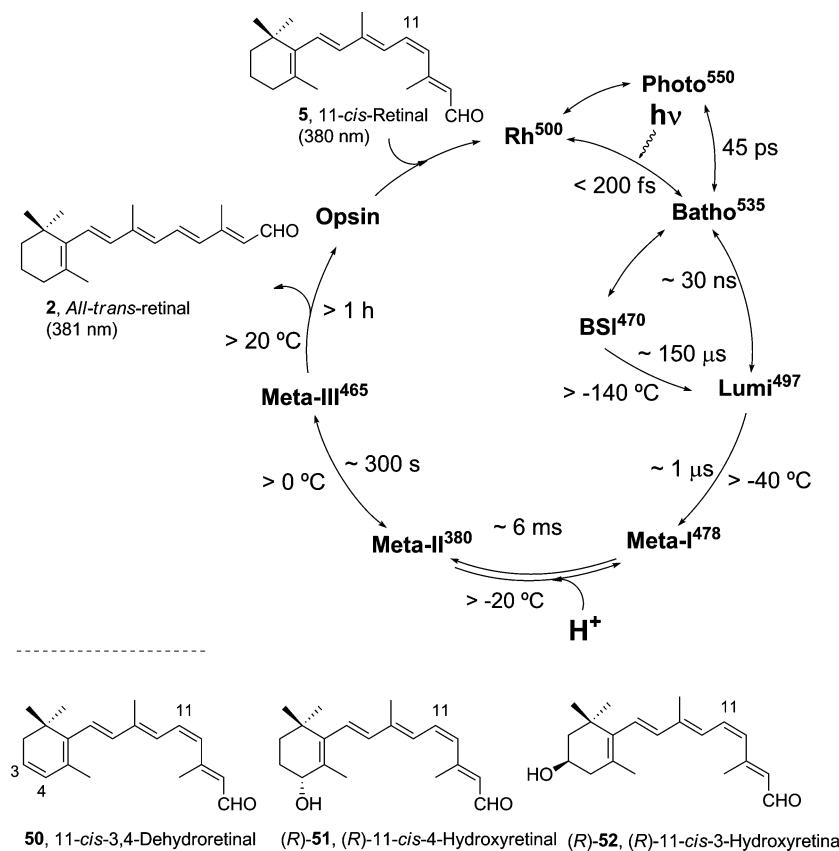


Figure 7. The visual cycle in vertebrates (values correspond to bovine rhodopsin) and alternative chromophores used by invertebrates.

Xanthorhodopsin¹⁵³ is a protein complex isolated from the extremely halophilic eubacterium *Salinibacter ruber* living in salt ponds, which contains the carotenoid saniliyanthin (a glycosylated carotenoid acylated with a fatty acid) and all-trans-retinal (**2**) in a molar ratio of about 1:1 (see section 10.1.3).¹⁵⁴ The X-ray structure of xanthorhodopsin shows a center-to-center distance between both chromophores of ca. 11 Å with an orientation of their molecular axes of ca. 46°. This structure explains the highly efficient energy transfer (between 30% and 50%) from the carotenoid to the retinal measured in the complex,¹⁵⁵ and the role of the carotenoid antenna extending the absorption of the bacterium to the green and blue spectral regions.¹⁵⁶

Archaerhodopsin-2 (aR2) is a trimeric retinal protein–carotenoid complex found in the claret membrane of *Halorubrum* sp. *aus-2* where it functions as a proton pump.¹⁵⁷ The protein shares about 56% sequence identity with bR and also utilizes the photoisomerization of all-trans-retinal (**2**) at the C13=C14 bond to pump protons. However, the absorption maximum of the chromophore in the unphotolyzed state is $\lambda_{\text{max}} \approx 550\text{--}570$ nm, and this difference with bR is due to the presence of the carotenoid bacterioruberin. The role of the carotenoid however appears to be primarily structural,¹⁵⁸ because it binds a crevice between the subunits of the trimer and increases the thermal stability of the complex relative to the monomer.¹⁵⁸ Halorhodopsin (HR) from *Natronomonas pharaonis* likewise crystallized as a trimer with the carotenoid bacterioruberin bound to crevices between the units.¹⁵⁹

4.2. Type II Opsins. The Visual Pigments, G-Protein-Coupled Receptors

Multicellular organisms that depend on the retinal chromophore for light absorption use 11-cis isomers.^{16c,160} The chromophore of most vertebrate visual pigments is 11-cis-retinal (**5**), but some fishes and amphibians use 11-cis-3,4-dehydroretinal (**50**) (Figure 7) as a second chromophore (porphyropsin). The visual pigment in salmon is 11-cis-retinal (**5**) in fresh water and the 3,4-dehydro analogue **50** in the ocean to capture more light during migration. Tadpoles use 11-cis-3,4-dehydroretinal (**50**), but in frogs the chromophore is 11-cis-retinal (**5**). Squid vision is based on (R)-11-cis-4-hydroxyretinal ((R)-**51**), and insects have (R)-11-cis-3-hydroxyretinal ((R)-**52**) as chromophore.^{109,161}

Visual pigments^{16c,160} belong to the 7TM-domain GPCR superfamily.¹⁶² Humans have three cone visual pigments (that absorb short, medium, and long wavelengths at $\lambda_{\text{max}} \approx 425, 530$, and 560 nm, respectively) for bright light vision and trichromatic color vision and one rod visual pigment, rhodopsin Rh (which absorbs at $\lambda_{\text{max}} \approx 500$ nm), responsible for dim-light vision.¹⁰⁹ Other activities of opsins have been described, and vertebrate Rh was found to function as a lipid flipase¹⁶³ and Rh in *Drosophila* larvae as a thermosensor.¹⁶⁴

The heterotrimeric GPCR rhodopsin is composed of the 7TM helices apoprotein opsin and the 11-cis-retinal chromophore¹⁶⁵ covalently bound as a PSB to Lys₂₉₆ of helix 7, with Glu₁₁₃ of helix 3 acting as counterion (see Figure 3).^{16a,102,160,166} The chromophore in rhodopsin and cone opsins acts in fact as an inverse agonist because in the dark it suppresses the activity of the receptor to an undetectable level. The protein–ligand complex shows a very low level of

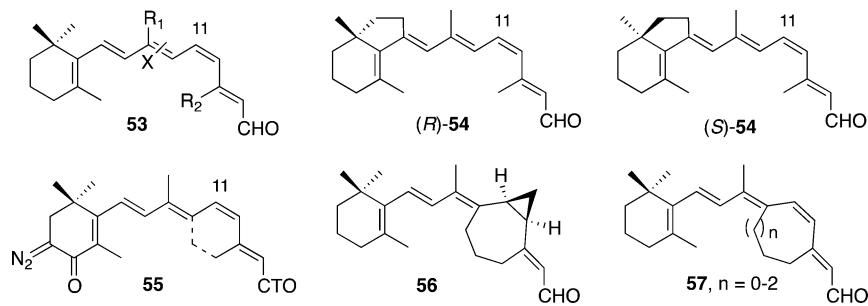


Figure 8. 11-*cis*-Retinal analogues for bioorganic studies of the visual process.

spontaneous thermal isomerization of the chromophore, a process that would produce the agonist-activated protein complex. Previous reports had indicated photoreceptor noise production due to thermal isomerization of the unprotonated Schiff base.¹⁶⁷ On the basis of kinetic measurements of thermal processes with Rh and selected mutants, it has been proposed that the reduction of the dark noise for dim-light detection is due to the stabilization of the complex by a hydrogen-bonding network coupled to the protonated Schiff base. This network has a minor effect on the photoisomerization.¹⁶⁸ Moreover, wild-type opsin catalyzes the isomerization of 11-*cis*-retinal (**5**) in solution without formation of the Schiff base, and this effect was proposed to be due to steric interactions in the binding pocket.¹⁶⁸

Light absorption triggers a photochemical isomerization of the chromophore to the all-*trans* configuration.¹⁶⁹ This primary photoisomerization event is an ultrafast (200 fs), efficient (quantum yield Φ of typically 0.65), and endergonic reaction (the first photoproduct intermediate bathorhodopsin stores 2/3 of the absorbed light energy).^{16a,c,160} Upon photoisomerization to all-*trans*-retinal (**2**) in bathorhodopsin (Batho, Figure 7), the conformationally distorted chromophore relaxes and generates the metarhodopsin II state (Meta) through a series of intermediates, and finally the chromophore is detached from the protein after hydrolysis of the photobleached product.¹⁷⁰ The active Meta II binds and transiently activates many copies of the heterotrimeric G-protein transducin,¹⁶² which modulates the transmembrane potential within the cell and induces an optical nerve signal that culminates in visual perception in the brain.

The three-dimensional structures of Rh^{102,171} (Figure 3) (a high-resolution solid-state NMR structure was also reported¹⁷²), Batho,¹⁷³ Lumi,¹⁷⁴ opsin in its free form¹⁷⁵ and in the G-protein interacting conformation,¹⁷⁶ the agonist-induced activated state,¹⁷⁷ the Meta II state,¹⁷⁸ and squid rhodopsin¹⁷⁹ have clarified many of the structural and functional properties of this light sensor protein.^{16b} The crystal structure of Metall state obtained by soaking crystals of opsin with all-*trans*-retinal (**2**) confirmed the presence of the undistorted chromophore bound to Lys₂₉₆ as a Schiff base and the change of orientation of the β -ionone ring relative to the conformation in the ground state and subsequent Batho¹⁷³ and Lumi intermediates.¹⁷⁴ This conformational change is the result of an apparent rotation of the chromophore along its main axis maintaining the 6-*s-cis*-conformation with a positive twist of the C6–C7–C8–C9 fragment.¹⁷⁸ However, the structure of a photoactivated constitutively active Rh triple mutant reconstituted with 11-*cis*-retinal (**5**)¹⁷⁷ has shown a 4.3 Å shift of the β -ionone ring (without C6–C7 rotation of the chromophore relative to Batho) toward the cleft between helix 5 and helix 6, which in its

turn induces a rotation of helix 6 and the displacement of Trp₂₆₅. These structural alterations favor the interaction with the G protein and the reorganization of water molecules of the hydrogen-bonding network in which some of the most conserved residues of the GPCR are involved. The structural differences with the Metall state suggest that this crystal structure might correspond to a trapped intermediate with the chromophore either entering or exiting the active pocket.

As in microbial opsins, 11-*cis*-retinal analogues have been important tools to facilitate the spectroscopic, structural, and functional studies of Rh,^{112b,121,180} especially before X-ray structures became available. A selection of retinal analogues for bioorganic studies of the visual process is depicted in Figure 8. Photoaffinity labeling with the nonisomerizable tritiated analogue **55** (locked by the six-membered ring, dotted lines) functionalized at the cyclohexenone ring with a diazoketone revealed the ground-state binding mode of the chromophore, and suggested an almost parallel disposition of the polyene relative to the membrane plane to maximize light capture.¹⁸¹ The same photoactivatable group in the nonlocked retinal **55** allowed one to examine the visual transduction path and led one to propose a flip-over of the β -ionone ring in the Batho to Lumi transition,¹⁸² which however was not confirmed in the crystal structure of these intermediates.^{173,174} The 6-*s*-locked analogues **54** provided useful information on the conformation adopted by the chromophore in Rh: because only **(R)**-**54** bound, and the artificial pigment exhibited about 80% activity of the native ligand and showed CD bands similar to those of native Rh, a negative absolute twist was proposed for the C6–C7 bond in the native chromophore (ca. -35° ; cf., -52.8° in the X-ray structure¹⁰²).¹⁸³ The artificial pigment formed with the (11S,12R) enantiomer of the bicyclic retinal chromophore locked by a seven-membered ring **56** provided insights into the ground state C12–C13 conformation. Spectroscopic and computational studies¹⁸⁴ suggested a positive twist with a C11–C14 dihedral angle of $+151.2^\circ$, which matches the experimental value (X-ray:¹⁰² $+151.6^\circ$). Therefore, the biologically relevant conformation of the flexible chromophore around the C6–C7 and C12–C13 bonds in Rh could be determined using artificial Rh's based on synthetic chromophores. Moreover, the slow binding of the *cis*-locked-7 ligand (**57**, $n = 1$) and the failure of the 8-membered ring homologue (**57**, $n = 2$) to form an artificial pigment led one to propose that 11-*cis*-retinal (**5**) enters the opsin binding pocket from the side close to the C5 and C13 methyl groups.¹⁸⁴

Artificial Rh's based on fluoro-,^{121c,185} side-chain-modified,¹⁸⁶ and ¹³C-labeled retinoids (general structure **53**, Figure 8)¹²² continue to offer valuable information on the photocycle and the structure of intermediates. Double-quantum solid-state NMR studies with ¹³C-labeled retinoids¹²² have provided

detailed C–C bond length data with a high spatial resolution (3 pm),¹⁸⁷ and information on the distortions of the chromophore in Batho and Metall.^{176b,188} In the active Metall state, solid-state ¹³C NMR spectroscopy has established the high polarization of the C=N bond, susceptible to hydrolysis, and the 6-s-*cis* conformation,¹⁸⁸ and confirmed that only small structural changes accompany photoactivation, as seen in the crystal structure of the Metall state.^{178,189}

Analysis of vibrational bands of the spectra taken between 200 fs and 2 ps at 50-fs resolution using femtosecond stimulated Raman spectroscopy¹⁹⁰ indicated that most of the rearrangement of the 11-*cis*- to all-*trans*-retinal chromophore occurs in the ground state, but extensive twisting keeps the overall shape of Photo (formally isomerized) and of Batho intermediates remarkably similar to that of the 11-*cis*-retinal chromophore. Thus, the geometrical changes take place in the ground potential surface during the Photo to Batho transition, using the energy of the photoexcitation stored in the first intermediate. The excited-state decay occurs through a CI mediated largely by hydrogen-out-of-plane wagging.¹⁹⁰ Ultrafast optical spectroscopy with sub-20-fs time resolution and spectral coverage from the visible to the near-infrared has allowed one to track wave packet dynamics leading to this CI.¹⁹¹

Computational studies,¹⁹² including hybrid QM/MM treatment,^{165,193} have also addressed the characteristics of the phoisoermerization process, the ultrafast conversion of photon energy into chemical energy, and the evolution of the excited state.¹⁹⁰ The accessibility of the CI between the potential energy surfaces of the ground and excited electronic states¹⁹¹ is reproduced by molecular dynamics simulations. The computations indicate that the chromophore uses the absorbed photon energy to drive minimal atomic displacement, mainly restricted to the polyene C9=C10–C11=C12 region, and reach the CI, where the average C11=C12 angle is -87.8° , in about 80 fs.¹⁹¹ The excited-state surface moves toward the conical intersection and onto the product with minor motions of the cyclohexenyl and Schiff base ends, but the C9=C10 torsional angle undergoes a large change (by 45°). Thus, the isomerization process involves only displacements on the C9=C10–C11=C12 region, in agreement with the structures derived from the femtosecond stimulated Raman scattering¹⁹⁰ and computational studies that predicted minimal motion of the chromophore (the bicycle-pedal¹³³ rather than the hula-twist¹⁹⁴ mechanisms).^{165,192c,d} The overall highly twisted structure can relax either to the all-*trans*- or back to the starting 11-*cis*-retinal configuration in the restricted binding pocket of the protein complex.¹⁹¹ Interestingly, computations predict that the electronic structure of the photoreceptor excited state can also be achieved thermally, thus providing an explanation to the thermal noise in rod photoreceptors.¹⁹⁵

Invertebrate rhodopsins, which differ from the vertebrate counterparts in the fact that they use an inositol-1,4,5-triphosphate signaling cascade as second messenger for signal transduction, showed by X-ray a structure very similar to that of Rh. Although the Rh polypeptide chain of squid (*Todarodes pacificus*) is about 100 amino acids longer than opsin in vertebrate Rh, it likewise folds into the classical 7TM structure.¹⁷⁹ In the crystal structure of squid Rh, the 11-*cis*-retinal (**5**) chromophore is bound to Lys₃₀₅ in helix 7 with Glu₁₈₀ as main counterion, and the retinal–lysine connection adopts a U-shaped conformation around Trp₂₇₄ in helix 6. The polyene chain, which is less distorted than that of Rh, is placed

perpendicular to the membrane plane, but the β -ionone ring is rotated about 90° relative to the polyene. The presence of water molecules in this structure suggests that the rearrangement of the hydrogen-bonding network is, as in vertebrate Rh, crucial for the activation of G proteins.¹⁷⁹

QM/MM computations on vertebrate and invertebrate rhodopsins have confirmed the greater flexibility of the binding site of the latter, in comparison with the more rigid vertebrate Rh binding pocket. Moreover, the invertebrate complex loses a greater amount of energy upon relaxation after the isomerization event, which was computed to take place most favorably by a bicycle-pedal mechanism.¹⁶⁵

Tuning of absorption maxima was traditionally considered to result from a combination of effects caused by the electrostatic interactions of the bound retinal and the neighboring residues of the apoprotein opsin including the PSB counterion and the polar amino acids (the external point charge model),¹⁹⁶ and by the distortions of the chromophore enforced by the protein binding pocket architecture.^{112a,197} The “opsin shift”¹¹² (for Rh this value is ~ 59 nm) quantifies the effect of the protein on the absorption maximum of the retinal-N-butylamine-PSB hydrochloride chromophore in MeOH (~ 441 nm). Recent studies have addressed the impact of ion solvation on the absorption maximum of the chromophore by gas-phase studies of the isolated PSB in an electrostatic ion storage ring. The chromophores are transferred to the gas phase by electrospray ionization and undergo irradiation by a laser pulse of defined wavelength.¹⁹⁸ Interestingly, it was found that the intrinsic absorption of the protonated all-*trans*-retinal-N-butylamine-PSB in these conditions is ~ 610 nm. Accurate CASPT2 calculations of the same model^{192b,199} predicted an excitation energy of 600 nm, close to this experimental value. A similar study of the Batho intermediate²⁰⁰ reproduced well the structure in the solid state and the spectroscopic properties of the chromophore.¹⁷³ Therefore, opsin proteins induce an increase (~ -110 nm for Rh and -42 nm for bR), rather than a decrease, of the chromophore transition energy. The unperturbed gas-phase absorption maxima values of the chromophore have been proposed to be a more accurate value to estimate the “opsin shift” of artificial retinal-based pigments.^{197,201}

The human cellular retinol binding protein II (hCRBPII, see Figure 2) has been engineered to encapsulate all-*trans*-retinal (**2**) covalently bound as a PSB inside the protein cavity. Depending on the type of mutations designed to alter the electrostatic environment within the binding pocket of the host protein, the absorption maxima of the artificial chromophore complexes could be finely tuned from 425 to 644 nm.²⁰² On the basis of complementary information obtained from X-ray structures, it was concluded that this wavelength regulation, spanning 220 nm, did not depend on conformational effects (C6–C7 bond rotation), contrary to what had been traditionally considered.^{112,196} However, maximal hypsochromicity was achieved by localizing the positive charge on the iminium nitrogen. Conversely, extreme bathochromic shifts were observed in mutants that had counter-anions interacting weakly with a PSB and functional groups that induced an even distribution of electrostatic potential across the polyene chain.²⁰²

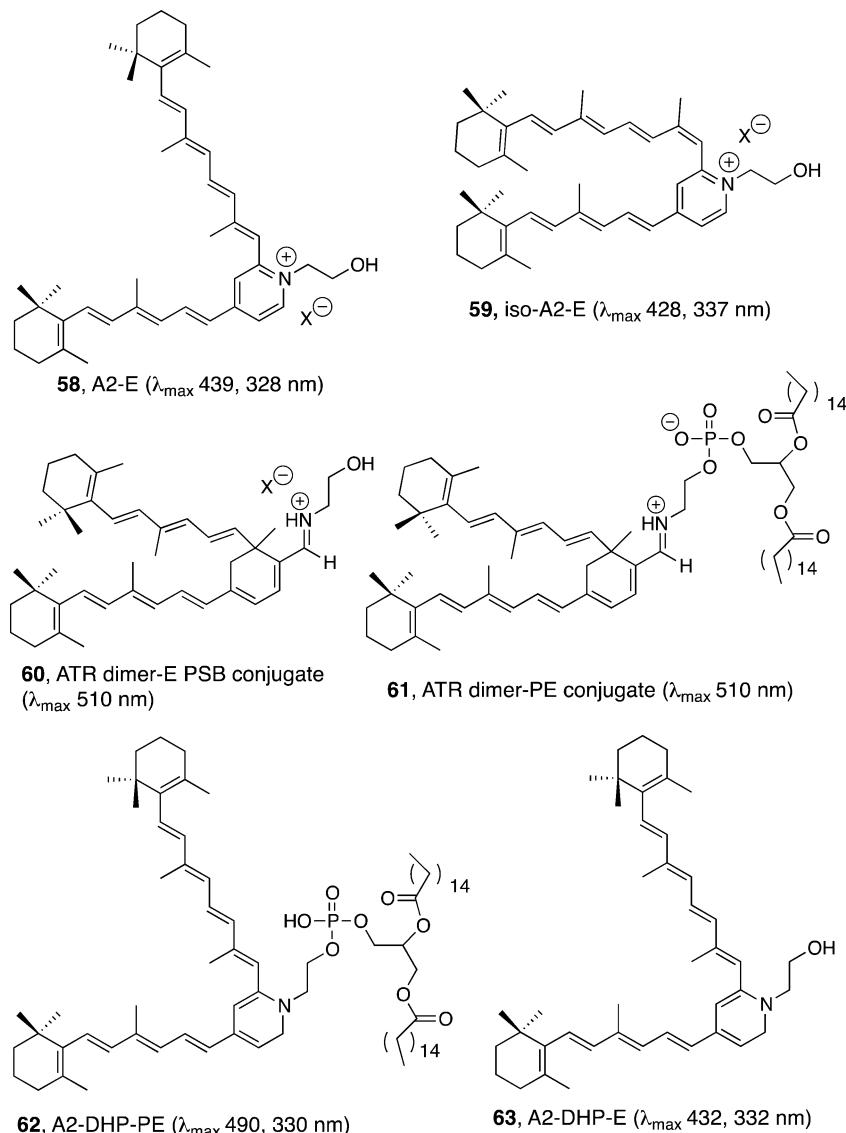


Figure 9. Structures of some bis-retinoid conjugates isolated from RPE cells, and absorption maxima of these fluorophores.

5. RETINAL LIPOFUSCIN PIGMENTS AND EYE DISEASES

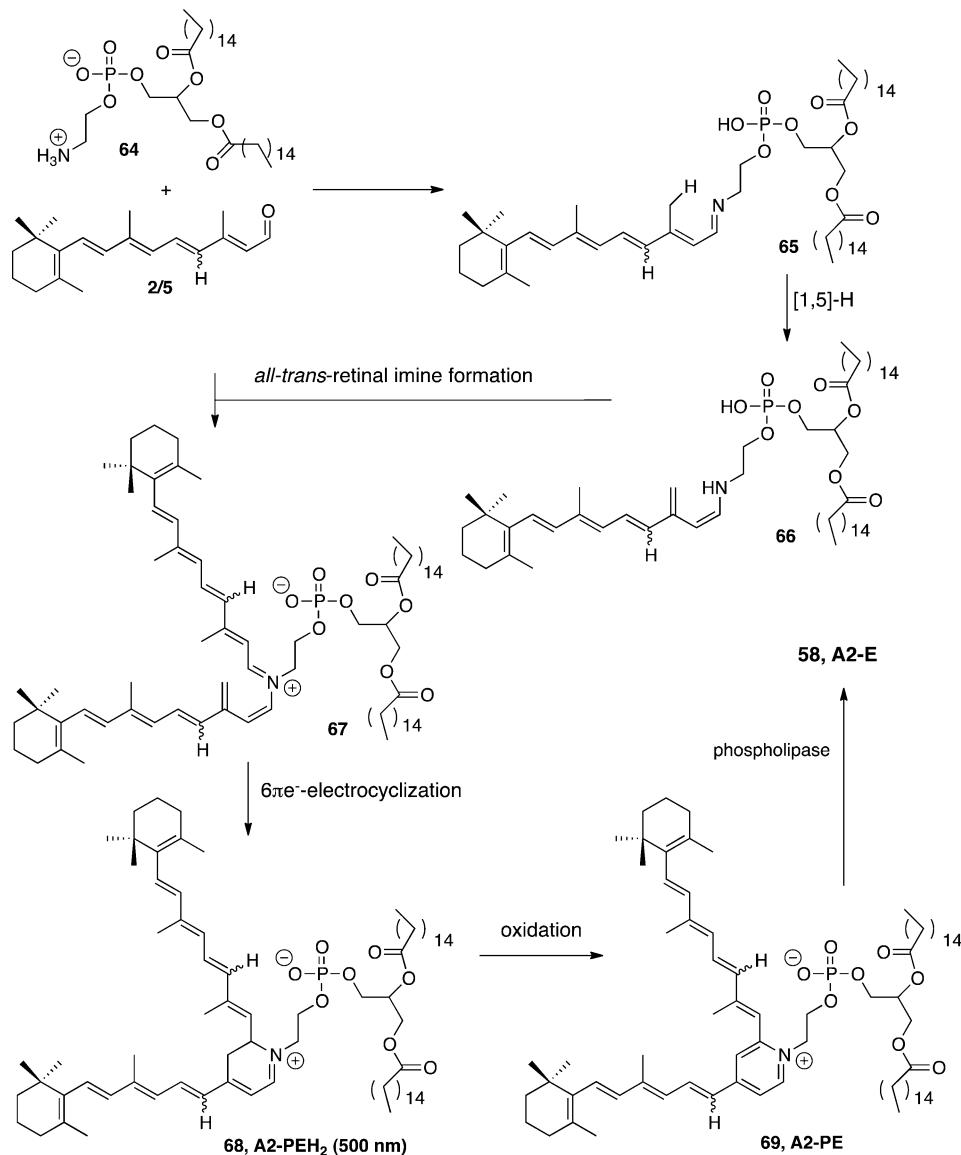
The so-called retinoid cycle²⁰³ accounts for the back isomerization of all-*trans*-retinal (2) to 11-*cis*-retinal (5) (Scheme 1).¹⁰⁹ Several steps are involved in this process. The all-*trans*-retinal (2) released upon rhodopsin photobleaching from the disk membrane to the cytoplasm is transported, as other vitamin A derivatives, by the multidrug resistance transport protein ABCA4, a member of the ATP-binding cassette transporters (ABC) protein family. All-*trans*-retinal (2) undergoes reduction by retinol dehydrogenases (RDH12/RDH8) to all-*trans*-retinol (1), which diffuses to the retinal pigment epithelium (RPE) and is esterified by LRTA and stored in the retinosomes. Isomerization of the all-*trans*-retinyl esters (8) to 11-*cis*-retinol (1) by RPE65 takes place before oxidation to 11-*cis*-retinal (5) by RDHS. The natural chromophore diffuses across the extracellular space to photoreceptors and regenerates the visual pigments.¹⁰⁹

Mutations in visual pigments are associated with diverse human diseases such as color blindness, retinitis pigmentosa, and congenital night blindness.^{16c,48,203b} ABCA4 is the only

gene associated with recessive Stargardt macular dystrophy, the most common form of early onset macular degeneration. It has also been linked to retinitis pigmentosa and cone rod dystrophy and to age-related macular degeneration (AMD), the leading cause of blindness in the elderly.²⁰⁴

In addition to mutations, the accumulation of autofluorescent age pigments (lipofuscin) in lysosomes of the RPE is a main contributor to eye diseases. Lipofuscin is a heterogeneous mixture of partially digested lipids and about 2% of proteins, which accumulates with age in several cells, in particular in the RPE.¹⁷ Bis-retinoids are the most abundant components of lipofuscin,²⁰⁵ and about 20 of them (those structurally characterized are shown in Figure 9) have been detected in eye tissues.¹⁷

N-Retinylidene-N-retinylethanolamine (A2-E, 58, Figure 9), the formal condensation product of two molecules of all-*trans*-retinal (2) and one molecule of ethanolamine (Scheme 5),²⁰⁶ is formed in the photoreceptor outer segment membrane from precursor A2-PE (dipalmitoyl- α -phosphatidylethanolamine, 69).²⁰⁷ Iso-A2-E (59), the 13-*cis* geometric isomer of A2-E (58) (Figure 9), has also been isolated from human RPE.²⁰⁸ The formation of a ca. 4:1 photostationary equilibrium mixture

Scheme 5. Hypothetical Biogenesis of A2-E (58) from All-*trans*-retinal (2) and Dipalmitoyl-L- α -phosphatidylethanolamine (64)

of the two isomers A2-E (**58**) and Iso-A2-E (**59**), together with minor amounts of other Z isomers, has been demonstrated after irradiation of each isolated bis-pyridinium salt in solution. A similar composition was present when eye extracts were used in the irradiation experiment.^{206b,208} The all-*trans*-retinal dimer-phosphatidylethanolamine (PE) conjugate fluorophore (ATR-PE, **61**)²⁰⁹ and the iminium ion derived from all-*trans*-retinal dimer and ethanolamine (ATR dimer-E PSB conjugate, **60**) were later characterized in human and mouse RPE cells (Figure 9).²¹⁰ Two additional lipofuscin pigments with dihydropyridine substructures, **62** and **63**, have been identified in human, mouse, and bovine retina and in a human model of recessive Stargardt disease (Figure 9).²¹¹ A2-DHP-PE (**62**) is more abundant in mice with a null mutation in *Abcr4*.²¹¹

It was hypothesized that the formation of A2-E (**58**) starts with dipalmitoyl-L- α -phosphatidylethanolamine (**64**) to produce the corresponding Schiff base **65** (Scheme 5). An unusual C-to-N [1,5]-H sigmatropic hydrogen shift from the allylic methyl at C20 to the imine nitrogen then generates a dienamine **66**, which undergoes condensation with another molecule of all-*trans*-retinal (**2**) to afford the polyeneiminium

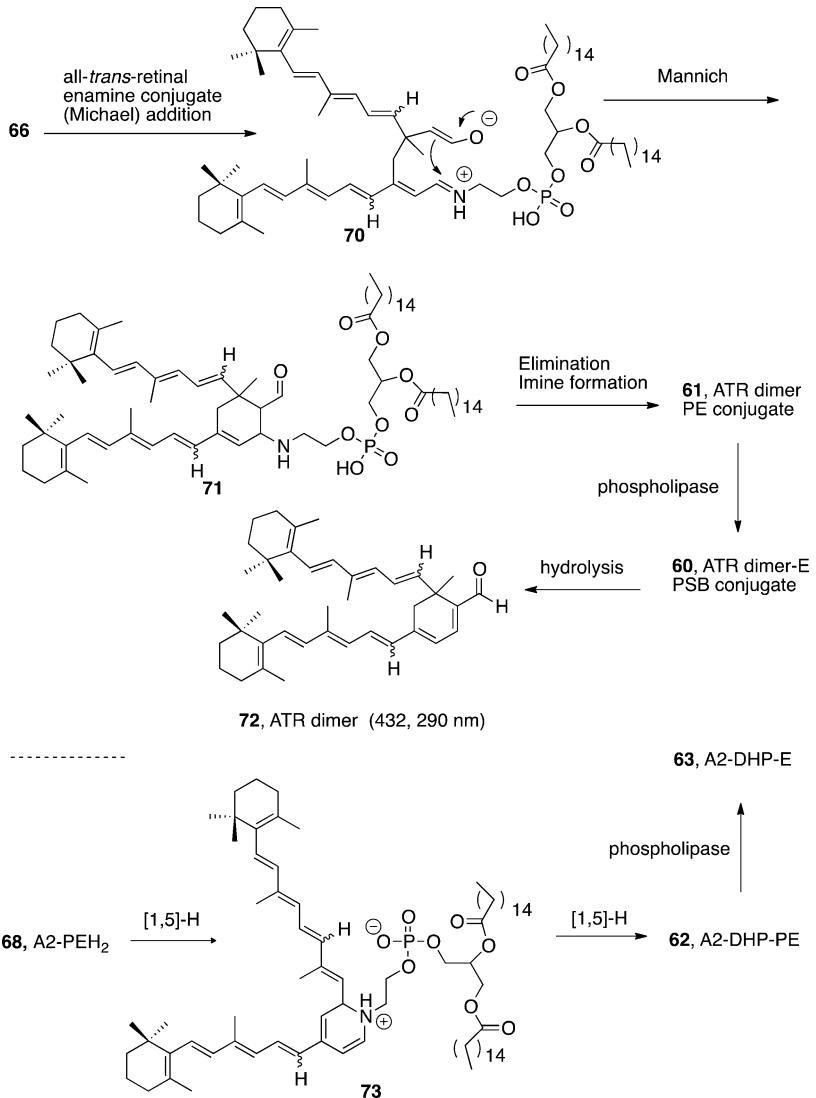
phosphate **67**. A 6 π e⁻-electrocyclic reaction generates the dihydropyridinium phosphate **68**, which evolves by oxidation and aromatization to **69**, and the lipofuscin pigment is released from **69** by the action of a phospholipase (Scheme 5).^{206a}

Formation of ATR-PE (**61**) could involve the same dienamine **66** (Scheme 5), which enters an alternative manifold involving a Michael-type conjugate addition to another molecule of all-*trans*-retinal, followed by intramolecular Mannich reaction of the adduct formed **70** to give **71**, elimination of the β -PDE group, and imine formation of the released amine with the carbonyl to afford **61** (Scheme 6).²¹²

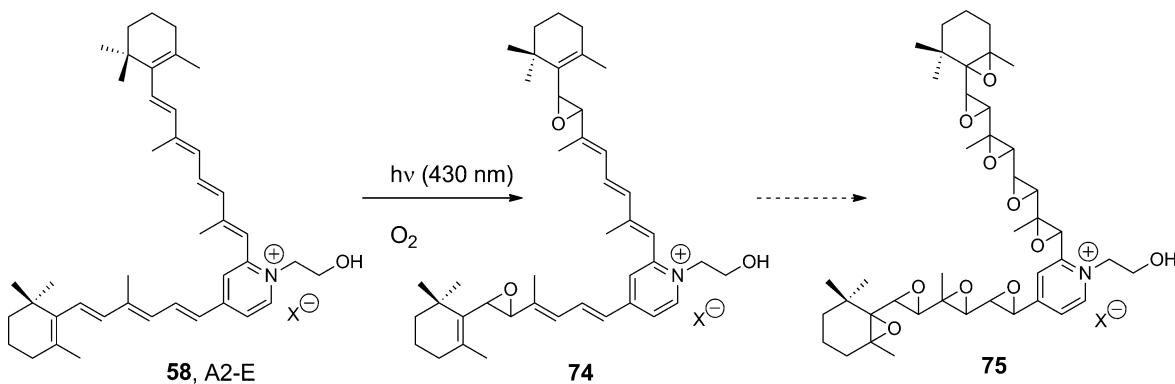
The PE conjugate A2-DHP-PE (**62**) could presumably be formed (Scheme 6) by isomerization of the dihydropyridinium zwitterion **68** (the same intermediate giving A2-E) by some of the available mechanisms (for example, two consecutive [1,5]-H shifts) to give the DHP isomer **73** that evolves to give the enamine **62** ($\lambda_{\text{max}} \approx 490$ nm). The action of a phospholipase produces A2-DHP-E (**63**) ($\lambda_{\text{max}} \approx 432$ nm).²¹¹

In support of the involvement of a [1,5]-H shift in the formation of lipofuscin pigments, it has been shown that the incorporation of all-*trans*-retinal labeled with deuterium at C20

Scheme 6. Proposed Biogenesis of Lipofuscin Pigments ATR-PE Dimer Conjugate (61), A2-DHP-PE (62), and A2-DHP-E (63)



Scheme 7. In Vitro Experiments Showing the Photoinduced Oxidation of A2-E



slowed the formation of dimers in wild-type rodents²¹³ and in mouse models of Stargardt disease.²¹⁴ As a consequence, it was proposed that the administration of a C20-d₃ labeled vitamin A derivative to patients may reduce lipofuscin formation and eye diseases associated with ABCR4 genetic defects.²¹⁴

A recent report claims that lipofuscin and its pigments such as A2-E (58) are formed from 11-cis-retinal (5) and accumulate

in the absence of light exposure.²¹⁵ Consistent with this finding, it has been reported that the nucleotide-binding domain 1 (NBD1) of ABC4 interacts preferentially and with high affinity with 11-cis-retinal (5) (and not with the all-trans-retinal isomer 2),²¹⁶ which suggests that the protein is involved in 11-cis-retinal translocation. Moreover, it was proposed that the generation of lipofuscin, and thus the pathogenesis of Stargardt

disease, might be related to defects on the import of 11-*cis*-retinal (**5**) into the rod outer segment (ROS) discs.²¹⁶ These findings might require the change of configuration of the starting retinal in Schemes 5 and 6, and perhaps incorporate a late-stage isomerization in this mechanistic picture.

The highly conjugated and charged pyridinium bis-retinoid structure of A2-E (**58**) was at first considered responsible for the photodynamic inactivation of photoreceptor human RPE cells through a combination of photosensitizing activity to generate harmful reactive oxygen species (ROS), phototoxic effects, and detergent-like action.^{206a} It was also discovered that A2-E (**58**) targets cytochrome oxidase (COX), inhibits oxygen consumption upon irradiation, and induces apoptosis in mammalian RPE cells.²¹⁷ During irradiation with blue light ($\lambda_{\text{max}} \approx 430$ nm), A2-E (**58**) undergoes photooxidation with singlet oxygen and produces the corresponding epoxides primarily at C7=C8 and C7'=C8', forming diepoxyde **74**, but also at other positions depending upon the duration of the irradiation, to finally afford the nonaoxirane **75** (Scheme 7).²¹⁸ This compound, like singlet oxygen, was found to induce cell damage. Further, the detergent-like activity of A2-E (**58**) was confirmed in various solvents.^{206a} Moreover, through competitive reversible inhibition of recombinant isomerohydrolase RPE65 in mice, A2-E (**58**) was found to inhibit the regeneration of 11-*cis*-retinal (**5**). This depletion may cause local starvation of this chromophore in the central retina and lead to receptor degeneration, as observed in the pathogenesis of Stargardt disease.²¹⁹

The unconjugated all-*trans*-retinal dimer (ATR, **72**), resulting from the elimination of PE from conjugate **61**, has also been characterized in human and mouse RPE cells (Scheme 6).²¹⁰ When irradiated at 430 nm, this compound was found to be a more efficient generator of singlet oxygen and more reactive with this species than A2-E (**58**). Solution experiments have provided evidence that **72** undergoes photooxidation of double bonds and demethylation, thus generating oxidized products. The structure of these photo-oxidized derivatives was tentatively assigned as the mono- and bis-endoperoxides and dihydrofurans on the ring C5–C8 fragment. These findings support the possible damaging effect of this chromophore on the photoreactivity of RPE lipofuscin.²¹⁰ Moreover, in Abcr^{-/-} mice, a model of recessive Stargardt macular degeneration, **72** was found in greater concentrations than A2-E (**58**).

A2-E (**58**) has in addition been characterized as a RAR α ligand by luciferase reporter assays, competitive binding, and transactivation studies.²²⁰ Moreover, A2-E (**58**) induced vascular endothelium growth factor expression in a human RPE cell line. The endogenous accumulation of A2-E sustained RAR activation in RPE cells and activated the expression of pro-angiogenic factors in vitro and in vivo.²²¹ For all of these deleterious effects, A2-E (**58**), and by extension the ocular bis-retinoids, should be considered as toxins, and as the only vitamin A derivatives not beneficial to human health.

The modulation of enzymes of the retinoid cycle can provide a means to control the flux of retinoids through the visual cycle.^{109,222} All-*trans*-retinoic acid (**3**) binds RPE65 and competes with all-*trans*-retinyl esters, the natural substrates of the enzyme. Moreover, isotretinoin (13-*cis*-retinoic acid, **76**, see Figure 16), a drug used for treatment and prevention of severe acne that inhibits retinol dehydrogenases, also binds RPE65.²²³ Thus, the ocular side effects of these drugs, night blindness episodes, could be due to the blockade of the RPE65-promoted

conversion of all-*trans*-retinyl esters (**8**) to 11-*cis*-retinol (**6**, Scheme 1). Retinoid-based drugs could also provide alternative therapies for certain retinal and macular degeneration disorders by limiting visual cycle turnover.²²³ Treatment of Abcr^{-/-} mice with isotretinoin was found to inhibit accumulation of lipofuscin,²²⁴ perhaps due to the inhibition of 11-*cis*-retinol dehydrogenase and reduction of the rate of 11-*cis*-retinal formation in the visual cycle. Retinylamine is a more potent and specific inhibitor of A2-E formation and also inhibits Rh regeneration.²²⁵ This compound is converted into the corresponding amides²²⁶ derived from fatty acids by LRAT (similarly to the conversion of retinol to retinyl esters).²²⁷

Finally, on the basis of the enhanced visual sensitivity reported as a side effect in patients undergoing porphyrin-based photodynamic therapy, a mechanism of excited state quenching from porphyrins to a rhodopsin acceptor molecule has been described by which night vision might be enhanced. Bleaching of bovine rhodopsin in the presence of porphyrin photosensitizers led to excitation of the visual pigment via electron or triplet-state energy transfer.²²⁸ This effect is reminiscent of the proposed triplet-state energy transfer from chlorophyll photosensitizers absorbing at 650 nm to the chromophores of the visual pigment of deep-see fish that absorbs at 545 nm, thus explaining their ability to sense light.²²⁹

6. ALL-TRANS-RETINOIC ACID, 9-CIS-RETINOIC ACID, AND THE RAR/RXR NUCLEAR RECEPTORS

Retinoids and rexinoids, as all other ligands of the nuclear receptor (NR) superfamily,^{19a} act as ligand-regulated *trans*-acting transcription factors that bind to *cis*-acting DNA regulatory elements in the promoter regions of target genes. These binding sites are frequently referred to as the NR “cistrome”,²³⁰ which comprises also binding sites that are formed due to the presumably cell-specific three-dimensional organization of chromatin. It is now clear that chromatin contains pre-existing interactions or forms such interactions in a factor-induced manner to generate a “chromatin interactome”. Recent mapping of RXR-RAR binding sites revealed that about 70% of these sites are in the extra or intergenic regions²⁴ and are likely to correspond to binding sites that interact with distant promoters through the three-dimensional structure, for example, through formation of chromatin loops. Consequently, we have to view receptor, or in general transcription factor, action in the context of a nonlinear complex and dynamic architecture of chromatin.

Conceptually, ligand binding does nothing else but modulate the communication functions of a receptor with its intracellular environment, which entails essentially receptor–protein and receptor–DNA or receptor–chromatin interactions. In this communication network, the receptor serves at the same time as intracellular sensor and regulator of cell/organ functions. Receptors are mediators of the information encoded in the chemical structure of a nuclear receptor ligand, as they interpret this information in the context of cellular identity and cell-physiological status and convert it into a plethora of receptor–protein and receptor–DNA/chromatin interactions, frequently involving the formation or recruitment of multisubunit complexes or molecular machineries with epigenetic, chromatin remodeling, and/or transcription initiating activities, which altogether results in the dynamic regulation of complex gene networks that constitute the physiological readout of RXR/RAR ligand action. To process input and output information, all nuclear receptors are composed of a modular structure with

several domains and associated functions. Main functions are the DNA-binding (DBD) and ligand-binding (LBD) domains, whose structures in the presence and absence of cognate DNA response elements and various agonists or antagonists have been determined with or without ligand,²³¹ as a ligand-bound domain heterodimer,²³² and as a full PPAR γ -RXR α -PPRE complex.²³³

The agonist-bound structure of the retinoid receptors ligand binding pocket (LBP)^{231b,d,234} is a compact fold of 11–12 α -helices and a short (s1–s2) β -turn arranged in three layers (“anti-parallel α -helical sandwich”) (Figure 10). The ligands are

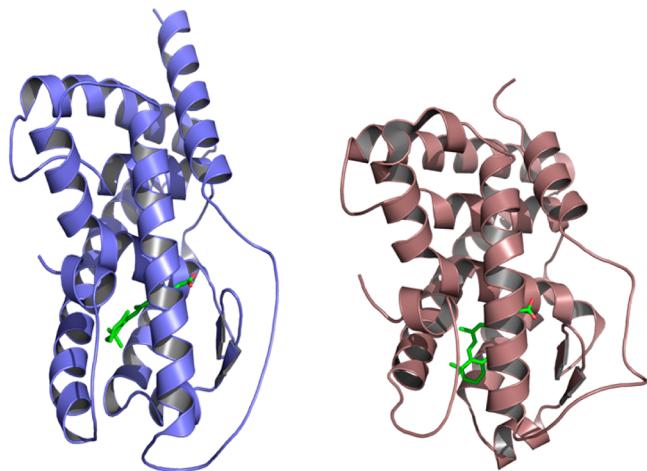


Figure 10. X-ray structures of the LBD of RAR γ complexed with 9-cis-retinoic acid (7) (PDB: 3lbd)^{231d} (left) and RXR α in complex with the same ligand (PDB: 1k74)^{234c} (right).

stabilized through extensive van der Waals contacts and by a network of ionic and hydrogen bonds involving their carboxylate moiety, a conserved arginine in H5, and water molecules. The “trans-activation helix” H12 seals the LBD of NRs, and the overall compact fold now exposes to the solvent some residues of H12, which are involved in coactivator interaction and thus trans-activation.

The LBD serves as dual input–output information processor, as ligand binding (input) induces allosteric changes of receptor surfaces that represent docking sites for subunits of transcription and/or epigenetic machineries, or enzyme complexes (output). The complexity of input and output signals, including, for example, post-translational receptor modifications such as phosphorylation,²³⁵ and their interdependence is not well understood, and no 3D structure of a full-length RAR–RXR heterodimer has yet been established.

It has been a major breakthrough of the past few years that we now recognize the enormous variety of communication processes that can be collectively or separately modulated by simply modifying the ligand structure, and we have begun to understand and pharmaceutically exploit the corresponding mechanisms.^{21,22,25b,236} The basis of nuclear receptor communication is their ability to provide surfaces for interaction in an allosterically controlled ligand-dependent manner. This communication reflects the cellular context, such as the ratio of coactivators and corepressors, which can decide whether a given ligand acts as agonist or antagonist.

In contrast to homodimeric receptors, retinoid receptors function essentially as RAR–RXR heterodimers, and thus their activity can be in principle modulated with two distinct ligands.

Indeed, the possibility of fine-tuning their activity through combinations of various classes of RAR- and RXR-specific ligands has been investigated both at the structural and at the functional levels. Several crystal structures of RAR–RXR LBD heterodimers bound to agonists or antagonists have been reported revealing that the overall structures of heterodimeric RXR and RAR do not differ significantly from that of their monomeric forms. Moreover, *in vitro*, specific ligands for each subunit are able to bind individually or in combination to their corresponding receptors and as such modulate RAR–RXR heterodimers. However, in the cellular environment containing coactivators and corepressors, retinoids alone are unable to do so.²³⁷ This so-called “RXR subordination” phenomenon is due to the inability of RXR ligands (rexinoids) to induce corepressor dissociation from heterodimers and thus to recruit coactivators because the binding site of the two types of coregulators is mutually exclusive. Therefore, RXR ligands cannot activate “nonpermissive” heterodimers (like RXR–RAR), which require the presence of ligands of the partner receptors for activation but can, on their own, activate “permissive” heterodimers (for example, PPAR–RXR), which are weakly associated with corepressors.

Thus, in RXR–RAR heterodimer, RAR agonists can autonomously activate transcription, while full responses to retinoids require the presence of RAR agonist ligands.^{237b} The strength of the overall association of RXR–RAR heterodimers with coactivators is dictated by the combinatorial action of RAR and RXR ligands, the simultaneous presence of the two receptor agonists being required for highest binding affinity.

6.1. Synthetic Retinoids

Stimulated by the multiplicity of activities of retinol and its downstream metabolites, and by the potential application of retinoic acids for the treatment of dermatological diseases, cancer, and metabolic diseases, medicinal chemists have carried out the design and synthesis of over 4000 retinoids, with the aim to discover more potent, selective, and safer analogues of the natural parent compounds for therapeutic applications.^{25,38a–c,238}

To overcome the limitation posed by the instability of polyenes to common laboratory conditions (light, heat, acids, bases), the design of new retinoids has focused on the use of (hetero)aryl rings as surrogate of some of the double bonds, giving rise to the group of aromatic retinoids. The so-called aritinoids show in general reduced configurational instability, stereochemical complexity, and conformational flexibility than the native ligands. In addition, from the pharmacological point of view, the orientation of the polar group with respect to the hydrophobic ring can be enforced in aryl derivatives to favor and/or improve the binding to the retinoid receptors (RAR and RXR). Figure 11 shows the conceptual evolution from the polyene structure of all-trans-retinoic acid (3) to the linked (hetero)aryl structures of the analogues that have replaced diene/triene moieties with aromatic rings. An early prototype was TTNPB (77), an stilbenoid with a dihydronaphthalene that showed potency similar to that of all-trans-retinoic acid (3).²³⁹ Naphthoic acids such as TTNN (79) and tetrahydroanthracenes represented by TTAB (78) are additional examples of aritinoids with alternative polyene fragments locked into aromatic rings. The methyl groups at the benzylic positions of these lead compounds block oxidative degradation.

The architecture of the ligand-binding pocket of RAR and RXR (Figure 10) determines that each receptor preferentially

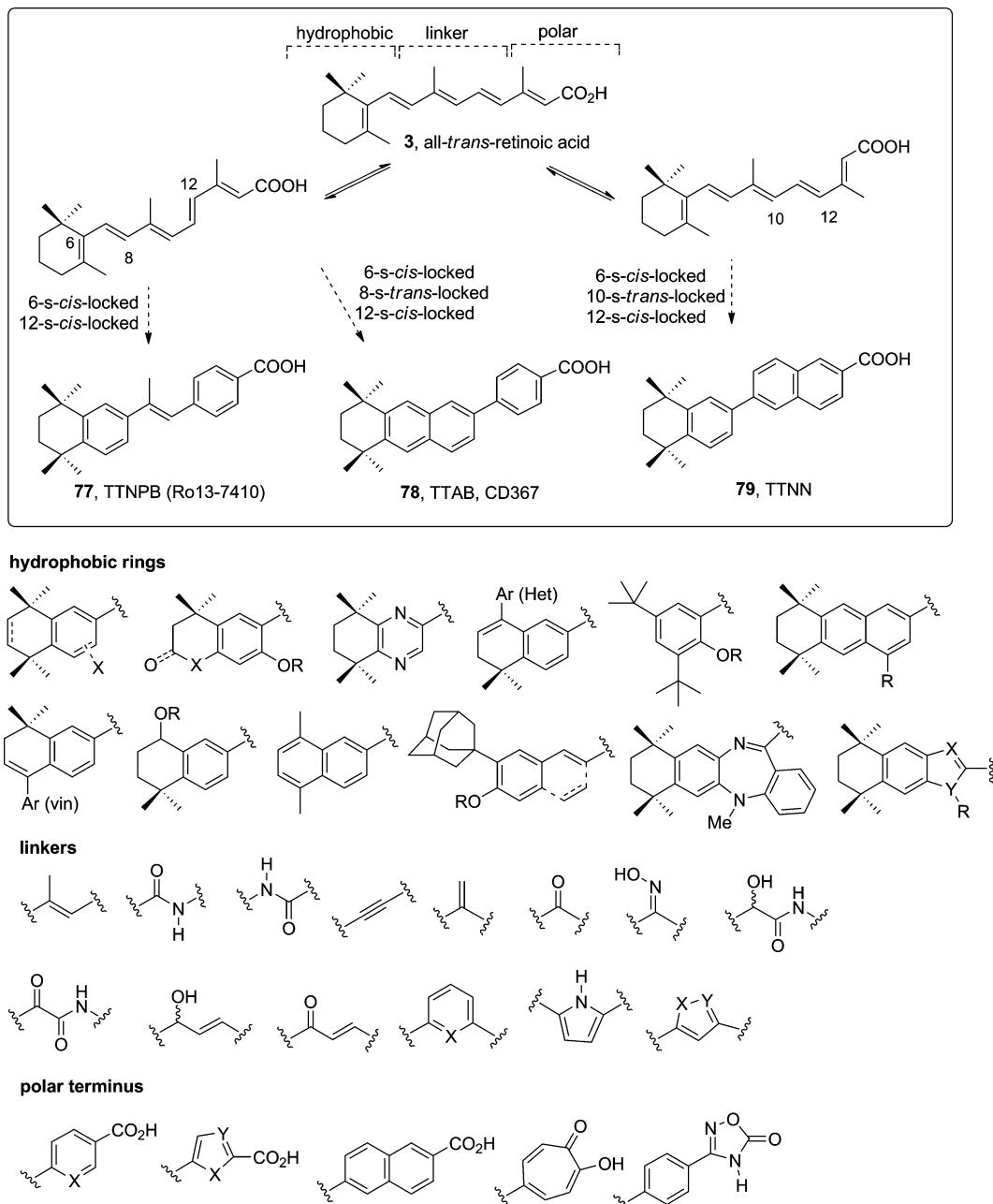


Figure 11. Structural evolution of all-trans-retinoic acid (3) to configurationally and conformationally locked retinoids by incorporation of double bonds into carbo(hetero)cyclic aromatic rings (arotinoids), and toolkit for construction of these compounds.

recognizes and binds topologically different ligand families. With the exception of conformationally flexible polyene ligands of *cis* configuration (i.e., 9-*cis*-retinoic acid 7 but not its all-*trans* isomer 3), retinoids and rexinoids are chemically distinct classes of compounds. In general, RAR ligands are elongated unsaturated carboxylic acids that fit into the linear I-shape of the receptor, whereas RXR ligands display a permanent or an induced twist that allow one to fill its L-shaped pocket (Figure 10).

Antagonists and inverse agonists are ligands endowed with bulky groups at defined positions that directly or indirectly²⁴⁰ produce steric clashes with the transactivation helix, induce the displacement of H12 from its active holo-position, and prevent coactivator recruitment.²⁴¹ The structural bases for the different

effects on H12 repositioning of inverse agonists and antagonists have been determined.^{231e}

6.1.1. RAR Ligands. Common retinoid scaffolds can be constructed by connecting the hydrophobic and polar termini with a linker using as a synthetic toolbox the building blocks shown in Figure 11.

Out of the many synthetic arotinoids reported, some have reached a privileged status by becoming chemical probes that modulate the activities of the retinoid receptors or because they are undergoing clinical trials. Those approved for therapy are shown in Figure 16. Representative structures of compounds that function as RAR agonists (81,²⁴² 83²⁴³), pan-antagonist (85),²⁴⁴ inverse agonist (86)²⁴¹ or subtype-selective agonists (80 is RAR α -selective;²⁴⁵ 82 is RAR γ -selective²⁴⁶), and

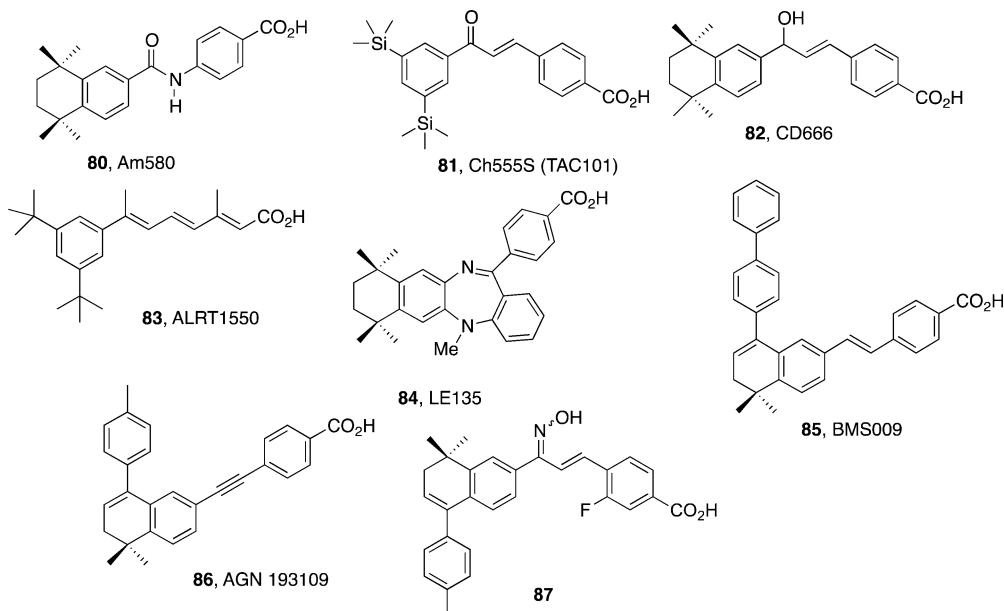


Figure 12. Selected RAR ligands.

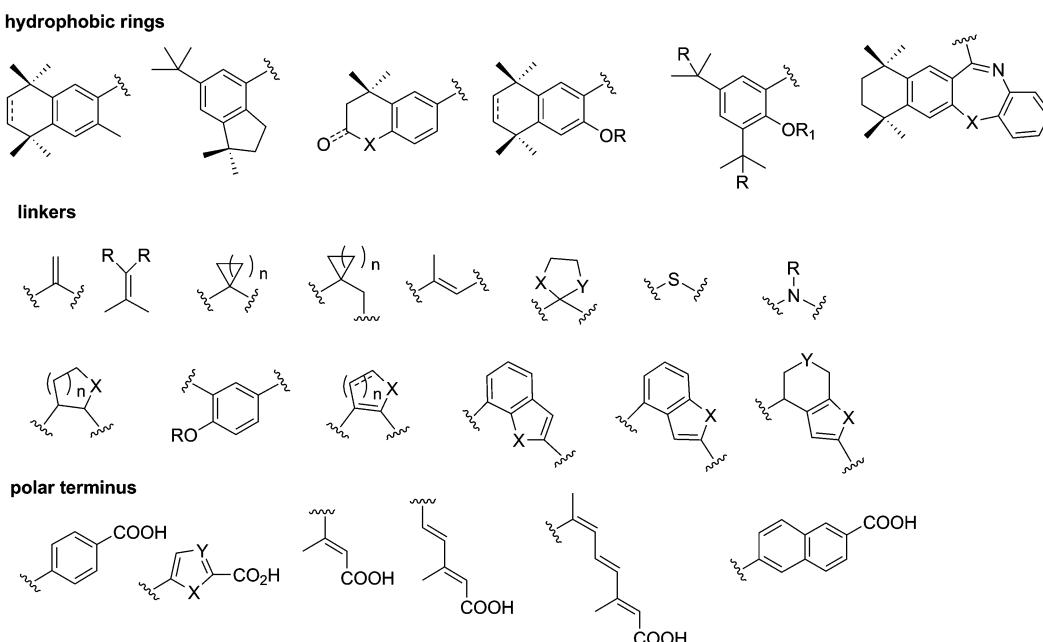
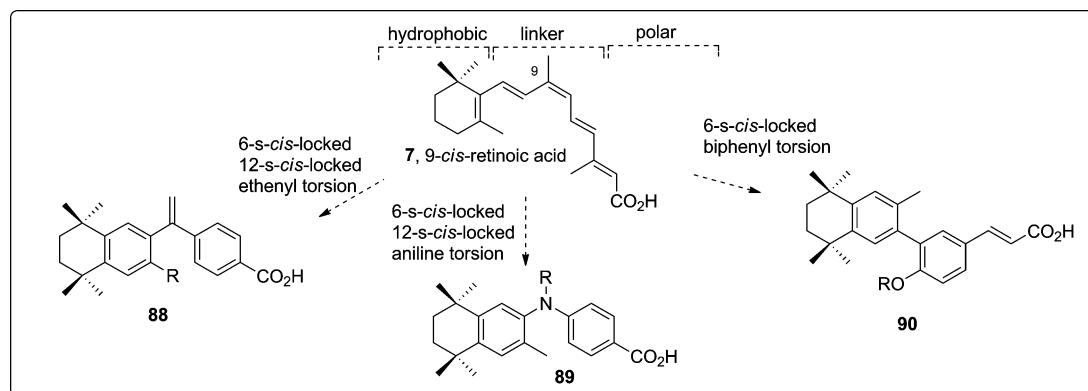


Figure 13. Structural evolution of 9-cis-retinoic acid (7) to configurationally and conformationally locked analogues (rexinoids), and toolkit for construction of these compounds.

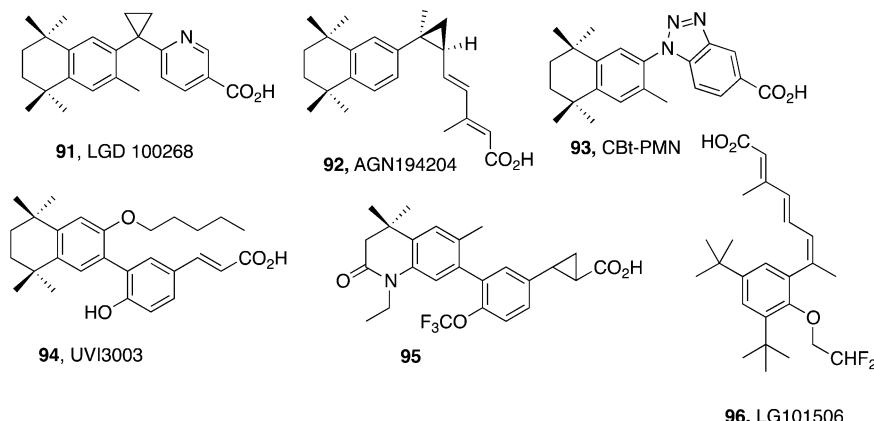


Figure 14. Selected RXR ligands with therapeutic potential.

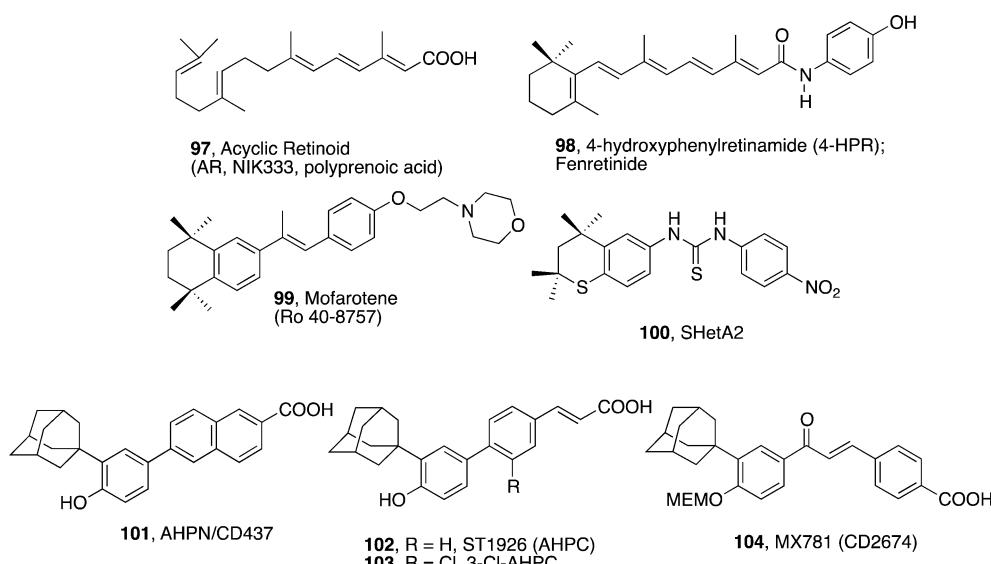


Figure 15. Selected atypical retinoids or retinoid related molecules (RRMs).

antagonists (**84** is RAR β -selective,²⁴⁷ and **87** is RAR γ -selective²⁴⁸) are depicted in Figure 12.

Am580 (**80**, a RAR α -selective agonist) was found to inhibit proliferation and induce apoptosis of mammary tumor oncogenesis in murine models (MMTV-neu and MMTV-neu-wnt1 transgenic mice) relevant to human cancer.²⁵⁰ Bis-trimethylsilylchalcone TAC101 (**81**) induced antiangiogenic activity and antimetastasis activity in liver,²⁴² showed chemopreventive effects on *N*-methyl-*N*-nitrosourea (MNU)-induced rat colon cancer,²⁵¹ and is undergoing clinical evaluation for the treatment of solid tumors.

6.1.2. RXR Ligands. Because of the L-shaped ligand binding pocket of RXR, which twists 9-*cis*-retinoic acid (**7**) around the C8–C9 bond in the RXR-bound conformation (Figure 10), most ligands that fit in the LBP show skeletal distortions. These structural alterations can be induced by a diversity of motifs, such as aryl rings connected by a saturated or a Csp² carbon (**88**), dibenzodiazepines, diarylamines (**89**), or biphenyl rings with *ortho* substituents (**90**).^{25b,236,252}

As explained for retinoids, the structure of compounds designed to interact selectively with RXR can be dissected into three structural units, the hydrophobic and polar termini and the linker (Figure 13).

Relative to the design of RAR ligands, RXR ligands display a greater variety of linkers, which is the region where the structural twisting is enforced, although they all share the presence of (hetero)aryl rings replacing some of the olefins of native retinoids. Representative RXR ligands are shown in Figure 14.

The cyclopropyl-substituted nicotinic acid LG100268 (**91**)²⁵³ has been evaluated preclinically for the treatment of metabolic diseases, since Zucker *fa/fa* rats treated with this rexinoid showed reduction in the food intake and body weight relative to control, and *db/db* mice (genetically defective in leptin signaling) showed increased insulin-stimulated glucose transport in skeletal muscle.²⁵⁴ The rexinoid with a *cis* bond locked by a cyclopropane at C6 and C7 of (*S,S*) configuration is the most potent and specific RXR agonist reported.²⁵⁵ AGN194204 (**92**) also acted as hypoglycemic agent in the *db/db* mouse model, but significantly increased serum triglyceride concentration in female Zucker diabetic fatty rats.²⁵⁶ The diarylamine scaffold CBt-PMN (**93**) conformationally constrained by the presence of a benzotriazole is a RXR partial agonist that showed antidiabetic effects in KK-A y mice, a model for type 2 diabetes, without inducing side effects associated with RXR full agonists.²⁵⁷ Antagonist **94** is a useful chemical tool to determine the effects of the partner ligands in

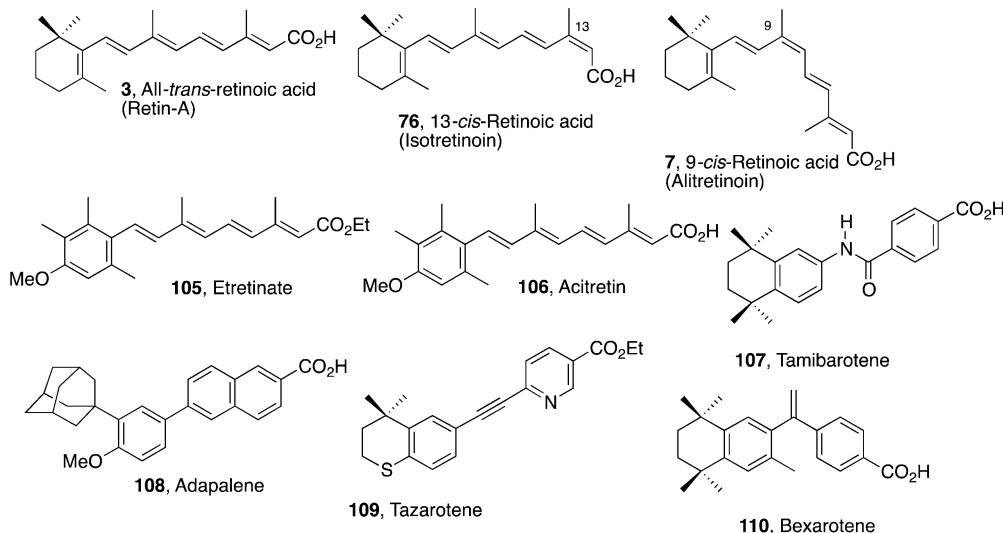


Figure 16. Retinoids used in therapy for dermatological and cancer indications.

heterodimers.²⁴⁰ The biaryl dihydro-[1H]-quinolin-2-one **95** is a selective LXR/RXR activator that showed good pharmacokinetic profile in rats.²⁵⁸ The trienoic acid **96** (LG101506) is a highly potent and selective PPAR γ /RXR heterodimer activator,²⁵⁹ and showed antidiabetic activity and induced the loss of body weight in animal studies without the undesired suppression of the thyroid hormone axis seen with full RXR agonists such as LG100268 (**91**).^{259b,260}

6.1.3. Retinoid-Related Molecules. Atypical retinoids or retinoid related molecules (RRMs, Figure 15) comprise a class of biologically active compounds that, despite being originally inspired by the structure of a natural retinoid or an arabinoid, exert their modulatory effects by mechanisms other than binding to and transactivation of the retinoid receptors.²⁶¹

Some of the RRMs, such as anhydroretinol (**28**), 14-HRR (**26**) (the activities of which have been described in section 3.3.1), and acyclic retinoid **97** (Figure 15), retain the polyene chain. Acyclic retinoid (ACR, **97**)²⁶² is currently undergoing clinical trials as an agent to suppress the recurrence of hepatocellular carcinoma (HCC) through its ability to induce apoptosis in premature HCC cells and its antiangiogenic effects due to inhibition of the VEGFR2MAPK pathway.²⁶³ The polyene is also preserved in the structure of all-trans-retinoic acid *p*-hydroxyanilide (4-HPR, **98**, fenretinide, Figure 15), which is the prototype of the peptidomimetic class of RRMs.²⁶¹ 4-HPR (**98**) is considered a chemopreventive agent based on the cumulative results of numerous in vitro and animal studies, as well as chemoprevention clinical trials, for cystic fibrosis, rheumatoid arthritis, acne, psoriasis, and Stargardt disease. Administration of 4-HPR prevented prostate tumor growth and metastasis in rats and induced apoptosis in human prostate cancer cells in vitro.²⁶⁴ Fenretinide increases urinary excretion of RBP4 (see section 3.2) leading to the normalization of serum levels of RBP4 in obese rodents (serum RBP4 levels are elevated in insulin-resistant mice and humans with obesity and type 2 diabetes²⁶⁵) through a mechanism that is independent of RXR binding and transactivation.²⁶⁵ Analogs of fenretinide that disrupt more potently the interaction of sRBP-TTR and sRBP receptor and hold promise for the treatment of type 2 diabetes have been developed.²⁶⁶ Mofarotene (Ro 40-8757, **99**, Figure 15) is an arabinoid with a morpholine heterocycle that inhibits the growth of various human cancers both in vitro and

in vivo, and protects the bone marrow from the toxic effects of cyclophosphamide and 5-fluorouracil (5-FU) in vivo. Mofarotene (**99**) induced strong antiproliferative and apoptotic responses in most established Burkitt's lymphoma (BL) cell lines as well as in primary BL cells.²⁶⁷ SHetA2 (**100**) (Figure 15) is the prototype of the flexible heteroarotinoids or FlexHet class of RRMs.²⁶¹ This diarylthiourea regulates cell growth and differentiation similarly to retinoids, but does not directly activate RARs or RXRs. SHetA2 (**100**) inhibited the growth of ovarian cancer xenografts without evidence of toxicity and targeted the mitochondria in malignant cells. Additional studies with animal models have demonstrated that SHetA2 does not induce teratogenicity or skin irritation as classical retinoids, and is currently undergoing preclinical development.²⁶⁸ AHPN (CD437, **101**, Figure 15) induced apoptosis in several cancer cell lines independently of RAR binding (AHPN is a RAR γ -selective agonist). Other members of the same class of adamantly arabinoids or AdArS²⁶¹ with cinnamic acid (ST1926, **102**)²⁶⁹ or chalcone **104** (MX-781)²⁷⁰ substructures are likewise potent inducers of cancer cell apoptosis. Complex mechanisms of action have been proposed for these compounds,²⁶¹ including the activation or blockade of signaling pathways,²⁷¹ activities that might be cell specific, and the binding to the orphan nuclear receptor small heterodimerization partner (SHP) in the case of 3-Cl-AHPC (**103**).²⁷² These RRMs have demonstrated activity against a number of tumors in nude mouse xenograft models and were found to prolong their survival.²⁶¹

Apocarotenoids, due to the close structural similarity to native retinoids, also function as signaling molecules.⁵⁴ For example, 14'-apo- β -carotenal (**19**) and 13-apo- β -carotenone (**20**, Scheme 2) were characterized as ligands of nuclear receptors (RXR, PPAR α , and PPAR γ),²⁷³ and similarly to 14'-apo- β -carotenoic acid (**21**), functioned as antagonists of all-trans-retinoic acid-induced transactivation of RARs.^{55b} 14'-Apo- β -carotenal (**19**) is a transcriptional repressor of RXR and PPAR responses,^{273a} whereas 13-apo- β -carotenone (**20**) is a RXR α antagonist.^{273b}

7. RETINOID THERAPY

7.1. Current Uses

Retin A (all-*trans*-retinoic acid, 3) is used for the topical treatment of moderate acne and for skin damaged by excessive sun exposure, reducing some wrinkles. Isotretinoin (13-*cis*-retinoic acid, 76) is indicated for the treatment of severe recalcitrant nodular acne. Etretinate (105) and Acitretin (106, Figure 16) are approved for the treatment of psoriasis, psoratic arthritis, and ichthyosis. Tamibarotene (benzamilide Am80, 107),²⁴⁵ a potent inducer of cell-differentiation,²⁷⁴ is approved in Japan for the treatment of refractory acute promyelocytic leukemia (APL). Adapalene (108)²⁷⁵ and tazarotene (109)²⁷⁶ (mixed RAR β/γ agonists) are topical retinoids approved for acne (adapalene, tazarotene) and psoriasis (tazarotene). Alitretinoin (9-*cis*-retinoic acid, 7) is used for the systemic treatment of refractory chronic hand eczema. The use of all-*trans*-retinoic acid (3) and As₂O₃ in combination (together with chemotherapy protocols primarily for postremission consolidation and maintenance therapy) for the therapy of acute promyelocytic leukemia (APL), in the past one of the most fatal acute leukemias, results today in the cure of more than 90% of patients.²⁸ Bexarotene (LGD1069, 110)²⁷⁷ is a therapeutic option for refractory cutaneous T-cell lymphoma (CTCL) and is undergoing clinical trials for the treatment of breast, lung, and colon cancer and other diseases caused by uncontrolled cell proliferation.²⁷⁸ Bexarotene has been found to reverse the effects of neurodegeneration in a mouse model of Alzheimer disease defective in the APP gene.²⁷⁹

7.2. The Therapeutic Potential of Retinoids and Rixinoids

7.2.1. Induction of Differentiation and Postdifferentiation Apoptosis. The cancer chemotherapeutic and cancer preventive action of retinoids and rixinoids have been reviewed previously.²⁸⁰ It is generally believed that the anticancer activity of retinoids is due to their ability to induce stem cell differentiation, as in the case of APL. However, other activities, such as postdifferentiation apoptosis involving activation of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) signaling,²⁸¹ contribute to therapeutic effects, at least in cell models and ex vivo experiments with leukemic blasts.

7.2.2. Induction of Rixinoid Apoptosis. Rixinoids can induce cell death through an entirely distinct signaling paradigm, a phenomenon referred to as “rixinoid apoptosis”.²⁸² In this case, the operative receptor is the permissive PPAR γ -RXR heterodimer, which induces NO through eNOS and iNOS activation. Importantly, this signaling is antagonized by growth factors through the MAPK pathway and is thus inactive under normal growth conditions. However, growth factor antagonists or limiting growth factor support activate death signaling in the presence of rixinoid or PPAR γ agonists. Interestingly, these effects have been confirmed with human breast cancer xenografts on nude mice and in ex vivo studies with leukemia patients’ blasts. A number of growth factor signaling inhibitors, such as gefitinib and herceptin, have been developed and are in therapeutic use as adjuvants to chemotherapy for the treatment of cancer, and several type 1 insulin-like growth factor receptor (IGF1R) inhibitors (blocking antibodies and tyrosine kinase inhibitors) are available. It will be interesting to test a combination of these inhibitors with RXR and/or PPAR γ agonists.

7.2.3. Extending the Therapeutic Potential of Retinoids to Non-APL Acute Myeloid Leukemia. As the

PML-RAR α fusion protein causes retinoid-sensitive APL, and as APLs expressing PML fusions with other proteins were refractory to all-*trans*-retinoic acid therapy, this therapy was believed to be applicable only to the rare cases of t(15;17)(22; q11.2–12) chromosomal translocation that generates the PML-RAR α fusion. However, it has been shown that a combination of a rixinoid with elevated levels of cyclic AMP (e.g., by phosphodiesterase inhibition) induced granulocytic differentiation and apoptosis of a large panel of all-*trans*-retinoic acid-insensitive acute myeloid leukemia (AML) patients’ blasts in ex vivo experiments, as in cellular AML models and all-*trans*-retinoic acid-resistant APL cells.^{281b}

More recently, an additional AML therapeutic paradigm has been described in which retinoic acid is combined with an inhibitor of the histone demethylase LSD1 (KDM1A).²⁸³ Apparently, the inhibition of this histone demethylase, which can remove methyl groups from mono- and dimethylated H3K4 (and H3K9), unleashes otherwise blocked retinoid signaling in AML blasts, leading to differentiation. These results suggest at the same time that retinoid signaling is defective in at least some types of AML cells and that LSD1 may contribute to AML pathogenesis by inhibiting the normal pro-differentiative function of all-*trans*-retinoic acid (3). Together, the above data further highlight the cancer therapeutic potential of retinoids and pave the way toward new combinatorial retinoid-based therapies for AML and beyond.

7.3. Cancer Chemoprevention

The cancer preventive action of vitamin A, described in 1925,^{26,27} was fully supported by a plethora of experiments revealing the suppression of carcinogenesis in vivo in experimental animals.^{14c,280a,c,284}

In recent years, two particularly important observations revealed significant conceptual progress toward the potential of retinoid for cancer prevention. One emphasizes the potential of combinatorial treatments for chemoprevention of colorectal cancer by targeting specifically premalignant cells for apoptosis.²⁸⁵ This study uses a combination of all-*trans*-retinyl acetate and TRAIL, which is known to induce apoptosis selectively in tumor cells.²⁸⁶ Note that expression of TRAIL or TRAIL receptors can be also induced via IRF1 by all-*trans*-retinoic acid in hematopoietic and breast cancer cells.^{281,287} Zhang et al. show that deficiency in the adenomatous polyposis coli (APC) gene and subsequent activation of β -catenin lead to the repression of cellular caspase-8 inhibitor c-FLIP expression through activation of c-Myc, thus sensitizing the cells for TRAIL-induced apoptosis. They observed that all-*trans* retinyl acetate upregulated the apoptogenic TRAIL receptors while repressing its decoy receptors in this system. Consequently, the combination of TRAIL and all-*trans*-retinyl acetate induced apoptosis in the APC-deficient premalignant cells without affecting normal cells in vitro. In addition, they showed that short-term and noncontinuous TRAIL and all-*trans* retinyl acetate treatment induced apoptosis specifically in intestinal polyps, strongly inhibited tumor growth, and prolonged survival in multiple intestinal neoplasms C57BL/6J-Apc(Min)/J (Apc(Min)) mice. The study revealed that TRAIL and all-*trans* retinyl acetate induce significant and specifically cell death in human colon polyps, thus providing a potentially selective approach for colorectal cancer chemoprevention.

The second insight started with the disappointing outcomes of a randomized phase-III cancer prevention trial of low dose 13-*cis*-retinoic acid (76) to prevent early stage head and neck

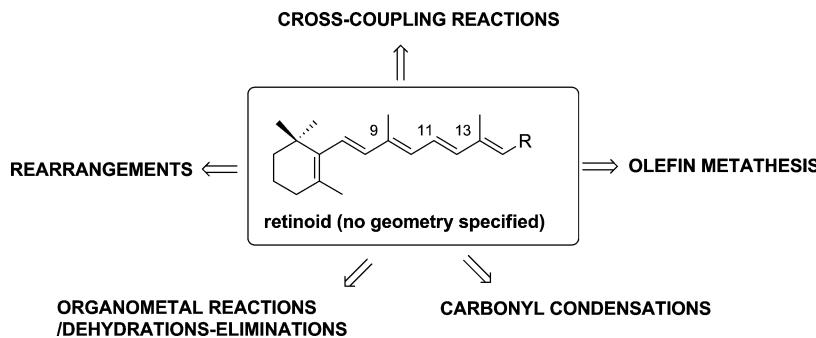


Figure 17. General strategies for the construction of the polyene skeleton of retinoids.

squamous cell carcinoma.²⁸⁸ In a follow up of this cohort of patients, Lee et al. decided to genotype 450 patients. The single-nucleotide polymorphisms (SNP) were analyzed for associations with second primary tumor or recurrence in patients receiving placebo to identify prognosis markers and further analyzed for effects of 13-*cis*-retinoic acid (**76**) in patients with these prognostic loci. Notably, 13 loci identified a subgroup of high risk patients in which the retinoid was protective; one of them was a SNP of the RXR α gene that marked a 3.33-fold increased risk in the placebo arm and a 38% reduced risk in the treatment arm.²⁸⁹ This study provides a proof-of-principle of the potential of pharmacogenetics to personalize cancer prevention.

7.4. Retinoid Toxicity and the Retinoic Acid Syndrome

Teratogenicity of retinoids is the most severe manifestation of toxicity.²⁹⁰ Craniofacial, cardiac, thymic, and central nervous system malformations (patterns that resemble those of animal studies)²⁹¹ have been recorded in human embryos of pregnant women with fetal exposure to isotretinoin.³⁰ These effects are ascribed to the activation of the retinoid receptors. In this regard, RXR ligands appear to be less teratogenic than RAR ligands, although they can potentiate the toxicity of the latter.²⁹²

Retinoid toxicity associated with retinoid supplementation can be manifested in drying, ulceration, and desquamation of the skin, anorexia, anemia, and bone loss. Hypervitaminosis also damages the liver through fibrosis and hepatic stellate cell activation, leading to cirrhosis. Bone loss in the elderly can lead to osteopenia and osteoporosis.²²²

Experience from therapy with acute promyelocytic leukemia (APL) patients showed that the so-called “retinoic acid syndrome” (also referred to as “differentiation syndrome”) occurs in approximately 25% of patients treated with all-*trans*-retinoic acid or As₂O₃ (as induction therapy for APL). Generally, the retinoic acid syndrome is associated with increasing leukocyte counts and is probably caused by the release of several cytokines by maturing blast cells. It gives a clinical picture of bodyweight gain, respiratory distress, serous effusions, and cardiac and renal failure. It also occurs in patients with relapsed or refractory APL who are treated with these agents. The risk of the syndrome is not a function of the dose of either of these agents, nor is it proportional to the white cell blood count. This syndrome is not observed when all-*trans*-retinoic acid and/or arsenic trioxide are used as consolidation or maintenance therapy for APL; it depends essentially on the presence of malignant promyelocytes.²⁹³

8. SYNTHESIS OF RETINOIDS

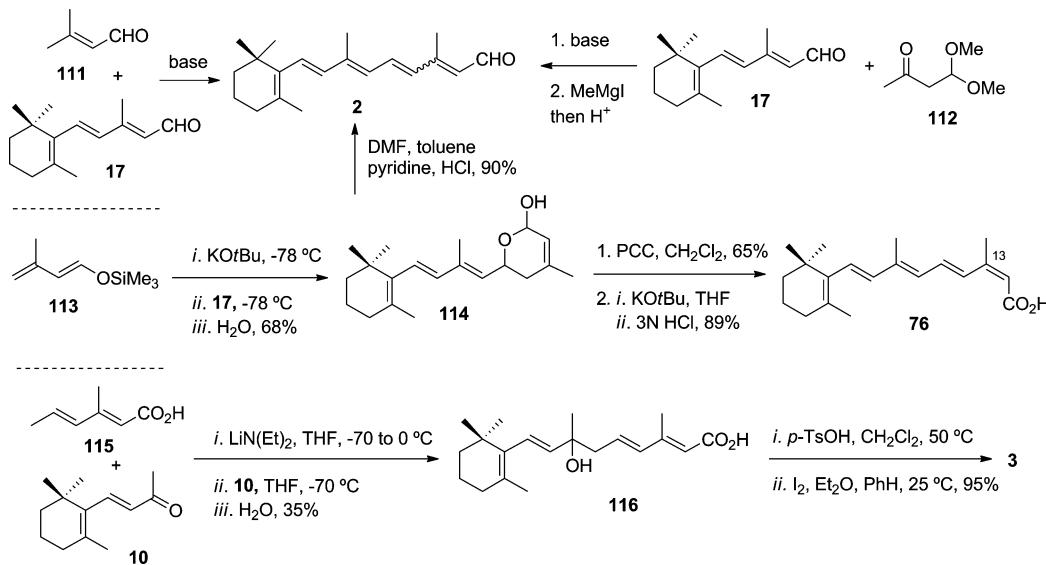
The formation of carbon–carbon double bonds is still considered a challenging task in the synthesis of unsaturation-containing molecules.²⁹⁴ Retinoids are oligoenes with trisubstituted-1,3-butadiene (isoprene) moieties. The number of possible diastereomers increases with the number of conjugated C=C bonds, and this stereochemical challenge can only be addressed using highly efficient and stereoselective synthetic procedures (Figure 17). Consequently, robust methods that yield geometrically homogeneous alkene and diene fragments²⁹⁵ and are compatible with the use of unsaturated substrates and reagents are in high demand for oligoene synthesis. The synthetic methods applied to the preparation of the biologically active retinoid isomers (all-*trans*, 11-*cis*, 9-*cis*, 13-*cis*) and related polyenes used for biological studies will be surveyed here. For the synthesis of arachinoids, the reader is referred to previous extensive reviews on the topic.^{38,39}

Two general approaches to oligoenes can be considered, which differ by the nature of the bond generated in the key step, that is, a double or single bond formation.²⁹⁶ Classical Csp²=Csp² bond forming processes have largely used carbonyl olefination reactions, such as the phosphorus-based Wittig and Horner–Wadsworth–Emmons (HWE) condensation, the sulfone-based Julia olefination, and the silicon-based Peterson olefination as well as their variants.²⁹⁷ The stereochemical outcome of these carbonyl condensations depends on various reaction parameters, but stereocomplementary versions of these reactions for the generation of the desired stereoisomer by proper choice of reagent/reaction conditions have been developed, as the Z-selective Still–Gennari²⁹⁸ and Ando²⁹⁹ modifications of the HWE reaction.

Over the past few decades, the Pd-catalyzed Csp²–Csp² bond forming reactions via cross-coupling of alkenyl metals (B, Sn, Zn, Si, Zr) and alkenyl electrophiles (halides and pseudohalides) has revolutionized the synthesis of dienes and polyenes.^{294b,300} Of relevance for the synthesis of oligoenes, the stereochemical outcome is in general encoded in the geometry of the coupling partners, and as a consequence this approach is more stereoselective than carbonyl olefination reactions.^{294b} Prior to Csp²–Csp² bond formation, alkenyl reagents or intermediates must be prepared regio- and stereoselectively via elementometalation of alkynes and elemento(halo)alkenylation of carbonyl compounds.

These two general approaches to oligoene skeletons by double- and single-bond forming reactions produce the new unsaturated fragment with positional selectivity in the construction step. Another category of reactions includes processes that generate the double bond(s) by base-induced elimination or dehydration after the alkylation, or the addition

Scheme 8. Aldol, Mukaiyama, and Extended Aldol Reactions for the Synthesis of the Retinoid Side-Chain



to carbonyl groups, of α -stabilized alkyl, alkenyl, or alkynyl organometallic reagents. Included in this group are the aldol, its vinylogous counterpart, and related reactions. The stereochemical outcome when using this construction tactic is more unpredictable, in particular if the oligoene is generated by elimination reactions or acid-induced rearrangements of allyl/propargyl alcohols, processes that produce in general mixtures of double-bond isomers at various positions of the polyene chain.

The olefin metathesis³⁰¹ catalyzed by metal carbene complexes (primarily of Ru and Mo) has recently been implemented as synthetic methodology for the challenging preparation of oligoenes and polyenes, including retinoids.

A last category of reactions includes the skeletal isomerizations and rearrangements of the already formed polyene, which in some cases can be of pericyclic nature, to afford the fully conjugated positional isomer. This group of reactions also generates mixtures of double-bond isomers of the final polyene, but selectivity has been achieved for particular substrates.

It is common practice in this field to label the building blocks of the synthetic scheme with the number of carbons $C_i + C_j$ ($i + j = 20$ for retinoids; and $i + j = 40$ or more complex $C_i + C_j + C_k + \dots + C_z$; $i + j + k + \dots + z = 40$ for regular carotenoids) they contribute to the final diterpene or tetraterpene skeleton. This terminology will be used where appropriate and should not be confused with the bond and carbon numbering ($C_x - C_y$).

Industrial synthesis of vitamin A is presently carried out by DSM (formerly Hoffmann–La Roche) and BASF with minor quantities produced by a few other companies.³ These industrial processes are based on the original procedures developed at Hoffmann–La Roche,³⁰² BASF,³⁰³ and Rhône–Poulenc (today Aventis)³⁰⁴ that have undergone optimizations in the last decades.³

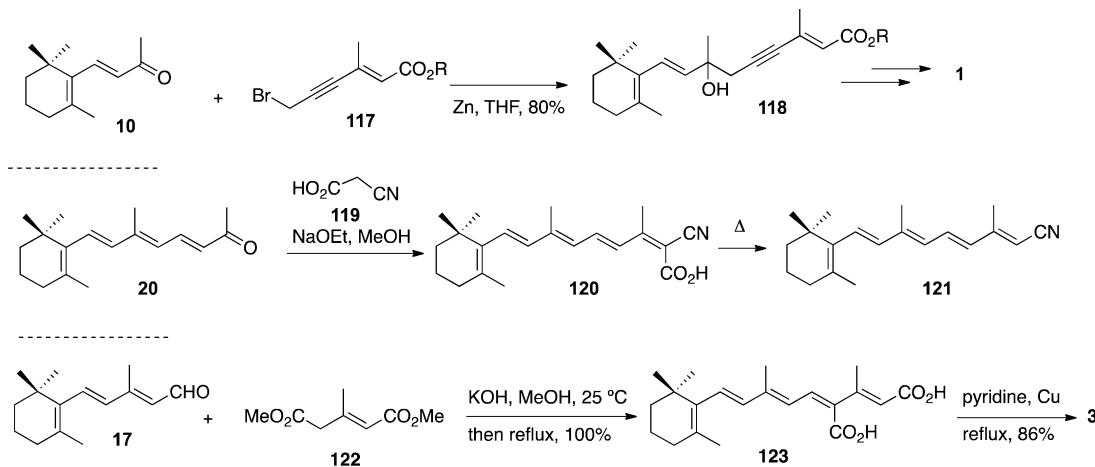
8.1. Addition of Metal Reagents to Carbonyl Groups/Dehydration, Elimination

The addition of metal reagents (enolates, extended enolates, alkenyl, alkynyl) to carbonyl groups followed by dehydration or derivatization/elimination is a general method for $C=C$ bond formation, but generally leads to mixtures of isomers that reflect their thermodynamic stability. In particular, the vinylogous aldol reaction to form the $C_{11}=C_{12}$ bond of retinoids using

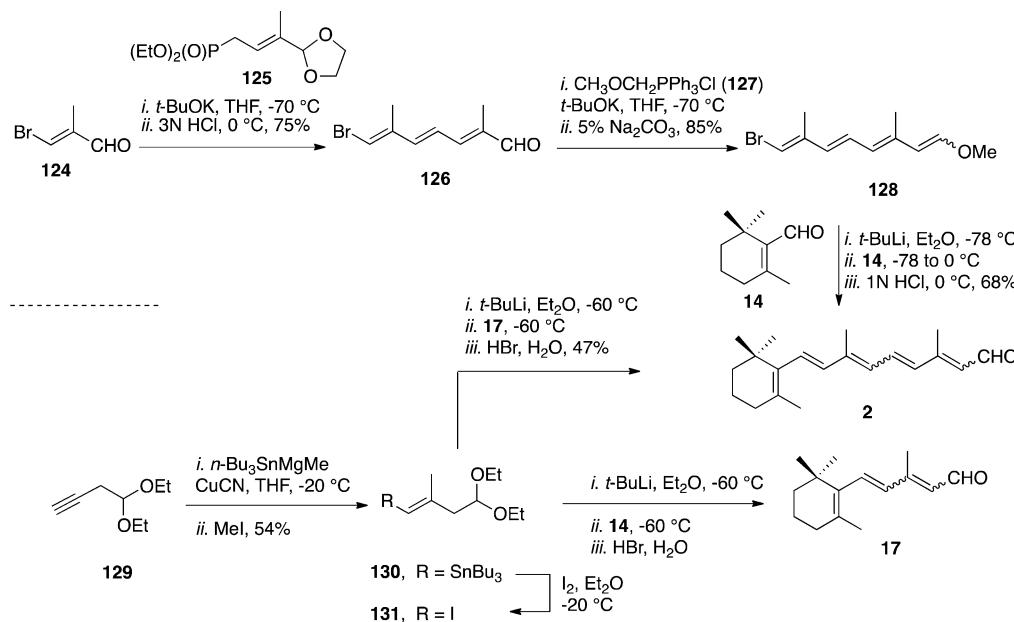
the $C_{15} + C_5$ approach has been thoroughly studied because it uses synthetic equivalents of the C_5 isoprenylation units, which are also useful for the synthesis of other terpenes.³⁰⁵

8.1.1. Aldol and Related Reactions. The direct aldol condensation of β -ionylideneacetaldehyde (17) and senecialdehyde (111) was the first recorded $C_{15} + C_5$ synthesis of vitamin A via all-*trans*-retinal (2), although the yields were very low as a result of the anticipated competing reactions.³⁰⁶ An alternative for the direct aldol condensation is the use of β -ketobutyraldehyde acetal 112 (Scheme 8). The reaction of the enolate of 112 with 17 and additional treatment of the resulting tetraenone with MeMgBr followed by deprotection/dehydration generated all-*trans*-retinal (2).³⁰⁷ The condensation of dienolates derived from 3-methylcrotonic acid and its esters (C_5 building blocks) with C_{15} -aldehydes has been the most utilized of these methods.³⁰⁸ However, in addition to the self-condensation of the enolate with the carbonyl compound, this vinylogous aldol strategy faces a number of problems for its application to the synthesis of retinoids, including the control of the regioselectivity of enolate attack (α vs γ vs ω), the regioselectivity of the addition in the case of unsaturated carbonyl compound (1,2- vs 1,4-addition modes), and the stereoselectivity of the subsequent dehydration step.³⁰⁵ Despite these potential complications, the adequate control of the reaction variables has led to the development of efficient synthetic methods for the construction of the retinoid side-chain. Under conditions of kinetic control (-78°C), the potassium enolate of prenol, generated from the preformed silyl enol ether 113 by treatment with catalytic quantities of KOtBu (which prevents retro-aldolization and inhibits the formation of the undesired γ -1,4 product as the intermediate alkoxide is *O*-silylated under these conditions), added to the C_{15} -aldehyde 17 in a γ -1,2-fashion.³⁰⁹ The potassium alkoxide intermediate then captures the released aldehyde yielding the cyclic hemiacetal 114. The final polyenal 2 was obtained in 90% yield, although as a mixture of four isomers in a 54:28:16:2 ratio, after treatment of the dihydropyran with acid (Scheme 8). If the dihydropyran is instead oxidized, the base-induced elimination/ring-opening provides 13-*cis*-retinoic acid (76). Regioselective addition of the more extended lithium trienediolates derived from hexa-2,4-dienoic acid (115) or dihydropyranones (C₇

Scheme 9. Miscellaneous Carbonyl Condensation Reactions



Scheme 10. Alkenyl-lithium Reagents in the Synthesis of the Retinoid Side-Chain



units) to β -ionone (**10**, C_{13}) under equilibration conditions afforded in low yield (35%) the 1,2- ω addition product **116**, which was subsequently dehydrated by treatment with acid and afforded all-*trans*-retinoic acid (**3**) as a mixture of double-bond isomers (Scheme 8).³¹⁰

The first industrial synthesis developed at BASF involved the extended Reformatsky-type addition to β -ionone (**10**) of the propargyl organometallic reagent formed from bromide **117**. The homopropargylic alcohol **118** could be transformed into vitamin A by either hydrogenation/elimination with *p*-TsOH or the reverse sequence, and final reduction with LiAlH₄.³¹¹

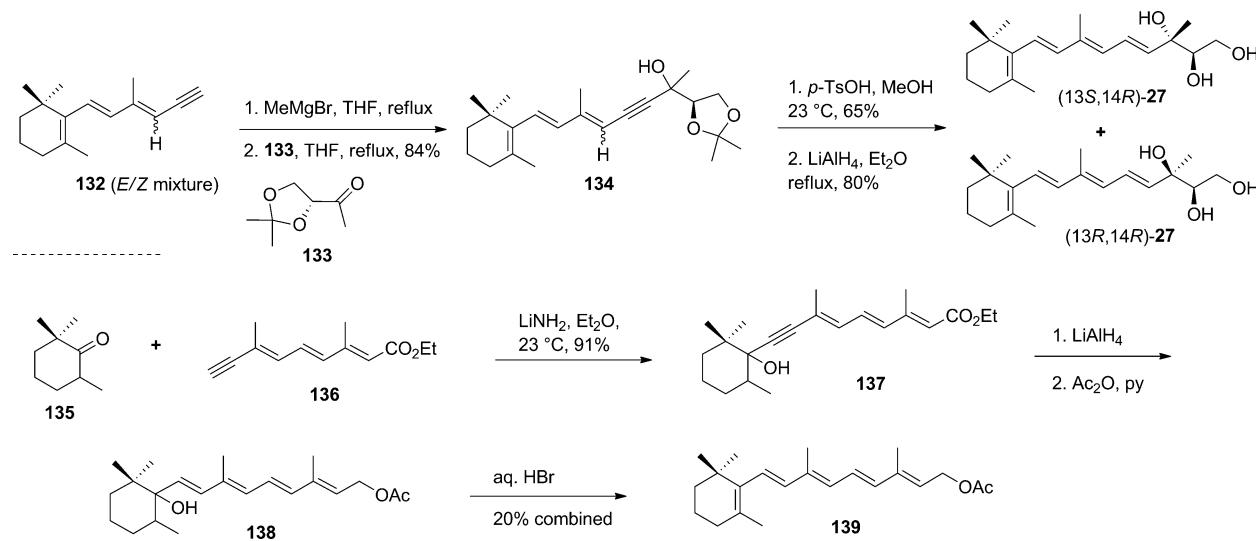
A Knoevenagel-type condensation of the C_{18} -ketone **20** and cyanoacetic acid (**119**) constructed the polyene skeleton of **120** and upon decarboxylation led to retinoylnitrile (**121**).^{303a,312} The condensation of **17** with methyl glutaconate (**122**) under alkaline conditions afforded C_{12} -carboxy derivative **123**, which was decarboxylated upon heating to reflux in pyridine in the presence of copper powder or copper salts to give all-*trans*-retinoic acid (**3**) (Scheme 9).³¹³

8.1.2. Alkenyl Organometals. The organometal compounds obtained by exchange of ω -halide-conjugated enol

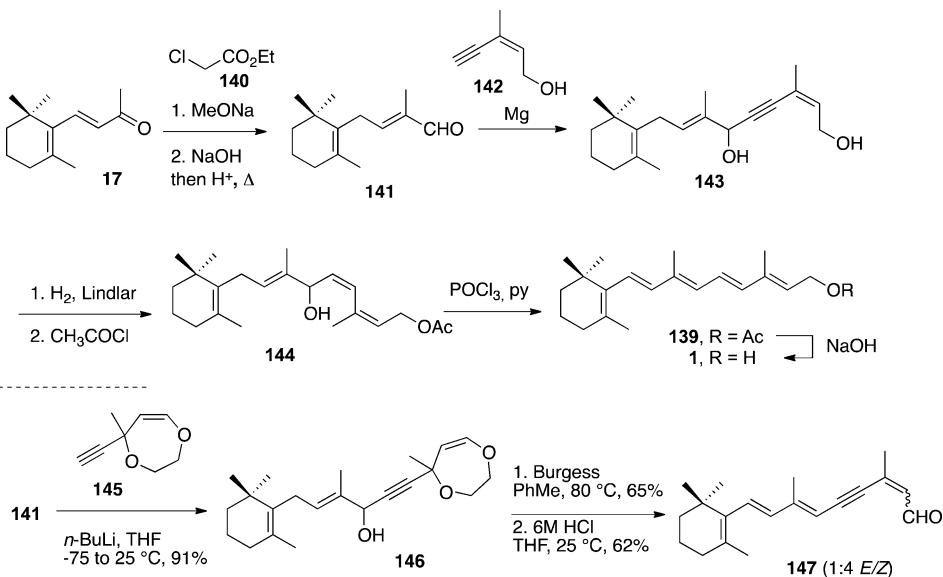
ethers or ω -halide-acetals can be considered synthetic equivalents of the extended metal enolates. They have been used for retinoid synthesis, in particular the C_5 prenal derivatives. Upon addition to aldehydes, the resulting allylic alcohols undergo dehydration under the same conditions employed for the hydrolysis of the silyl enol ether or acetal, unmasking the unsaturated carbonyl compound. In a $C_{10} + C_{10}$ approach to retinal, the vinyl organometallic reagent prepared by bromine–lithium exchange of **128** reacted with the C_{10} aldehyde β -cyclocitral (**14**) to afford the intermediate enol ether, which, without isolation, was hydrolyzed to give a mixture of retinal isomers in 68% combined yield. Compound **128** was obtained from methoxymethylenetriphenyl phosphorane and bromotrienal **126**, which is the condensation product of **124** and **125** and release of the protecting group. The C_{10} -vinyllithium reagent thus becomes a synthetic equivalent of ω -lithio-dehydrocitral, another extended vinylogous aldol anion (Scheme 10).³¹⁴

In addition, dehydration to retinal can be promoted from the alcohol obtained by reaction of an aldehyde with a C_5 γ -vinyllithium reagent³¹⁵ derived from halogen-metal exchange of

Scheme 11. Addition of Alkynylorganometals (and 1,2-Dehydration) in the Synthesis of Retinoids



Scheme 12. Addition of Alkynyl-lithium Reagents to Aldehydes and 1,4-Dehydration Reactions in the Synthesis of the Retinoid Side-Chain



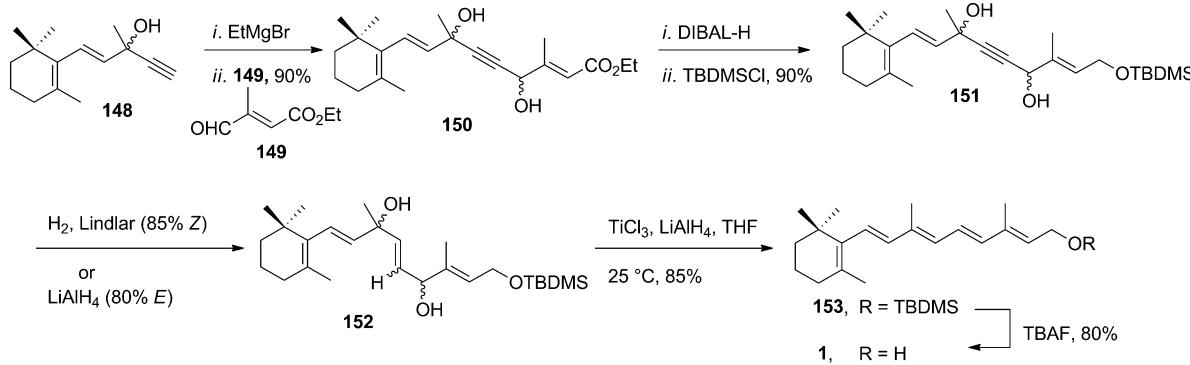
the vinyl iodide 131. This compound was obtained by stereoretentive tin–iodine exchange of a γ -stannyl- β,γ -unsaturated acetal 130, which results from the stannylcupration–alkylation of the homopropargylic acetal 129. The reaction of the vinylolithium derivative with C₁₀-aldehyde 14 afforded C₁₅-aldehyde 17, which underwent another chain extension with the lithium derivative of 131 to furnish a mixture of retinal isomers (68% *E*) in 47% overall yield after treatment of the condensation product with HBr (Scheme 10).

8.1.3. Alkynyl Organometals. Several alternatives to obtain the conjugated polyene from the propargyl alcohols obtained upon addition of Csp–M reagents to carbonyl compounds have been developed since the early industrial synthesis of vitamin A (C₁₄ + C₆ route) at Roche (see Scheme 12).³⁰²

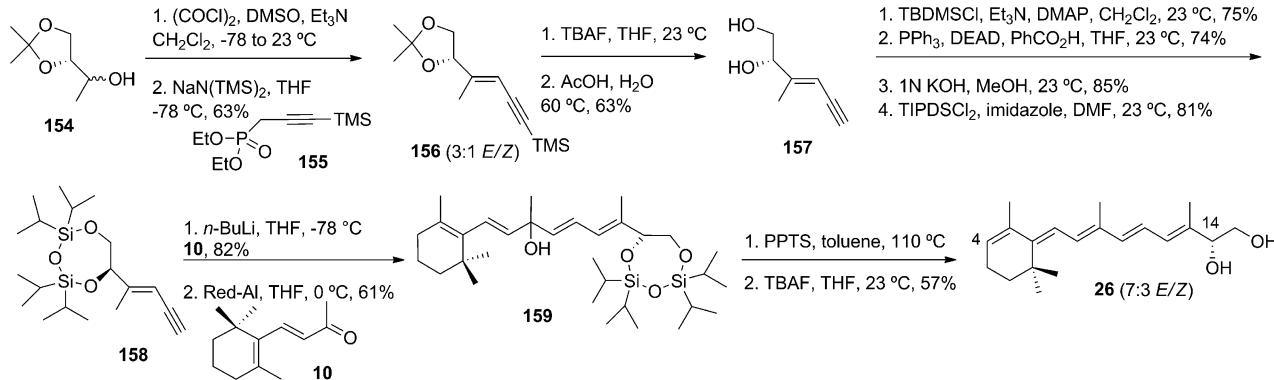
In the simplest case, the propargyl alcohol is reduced to the desired allyl alcohol, as in the synthesis of DHR (Scheme 11). The preparation of the (13*R*,14*R*) and (13*S*,14*R*) diastereomers of DHR 27 used ketone 133, available from D-glyceraldehyde

acetone, as chiral, nonracemic starting material (Scheme 11).⁷⁶ The addition of the Grignard reagent derived from trienyl 132 (a mixture of isomers at the trisubstituted double bond was used) to ketone 133 afforded a mixture of the four diastereomers of 134, which were separated by HPLC. The mixture of trans isomers of propargylic alcohol 134 was deprotected with *p*-TsOH, and the triple bond reduced with LiAlH₄ to provide the diastereomers at C13, compounds (13*R*,14*R*)-27 and (13*S*,14*R*)-27. The absolute configuration of the new stereocenter was secured by application of the exciton chirality coupling method to the experimental CD spectrum of a derivative.⁷⁶ However, comparison with the CD spectra of the natural sample (natural DHR was found to exist as a mixture of diastereomers in a 5:4 ratio) was inconclusive for configurational assignment. The small amounts obtained from the cell culture, and the dependence of the enantiomeric ratio with the culture conditions, precluded attempts to establish the absolute configuration of the natural compound. Thus, the absolute configuration of natural DHR (27) remains unknown. It was

Scheme 13. Low-Valent Titanium-Induced Double Reductive Elimination of But-2-ene-1,4-diols in the Synthesis of Retinoids



Scheme 14. “Ex-Chiral Pool”-Based Synthesis of 14-HRR (26) Using Extended 1,6-Dehydration



proposed that DHR (27) originates from the opening of the product of enantiospecific epoxidation of all-*trans*-retinol (**1**) bound to CRPB. Other unbound retinol molecules could likewise undergo epoxidation, and therefore the optical purity of the epoxide would depend on the biochemical status of the cell. Furthermore, although DHR (27) is converted into 14-HHR (26, Scheme 16) on mild acidic treatment, this conversion is not observed under physiological conditions, and DHR (27) can be considered an end-product of retinol metabolism with signaling properties.⁷⁶

A synthesis of vitamin A acetate (**139**)³¹⁶ includes a final dehydration of allyl alcohol **138**. The sequence starts with the addition to 2,2,6-trimethylcyclohexanone (**135**) of the trienynyl anion derived from the treatment of **136** with LiNH₂ to generate propargylic alcohol **137** as a 2:1 mixture of diastereomers in 91% yield (Scheme 11). Without separation, the mixture was treated with LiAlH₄ and then the primary alcohol was converted into the acetate **138**, which was dehydrated with aqueous HBr to provide **139** (20% combined yield from **137**).³¹⁶

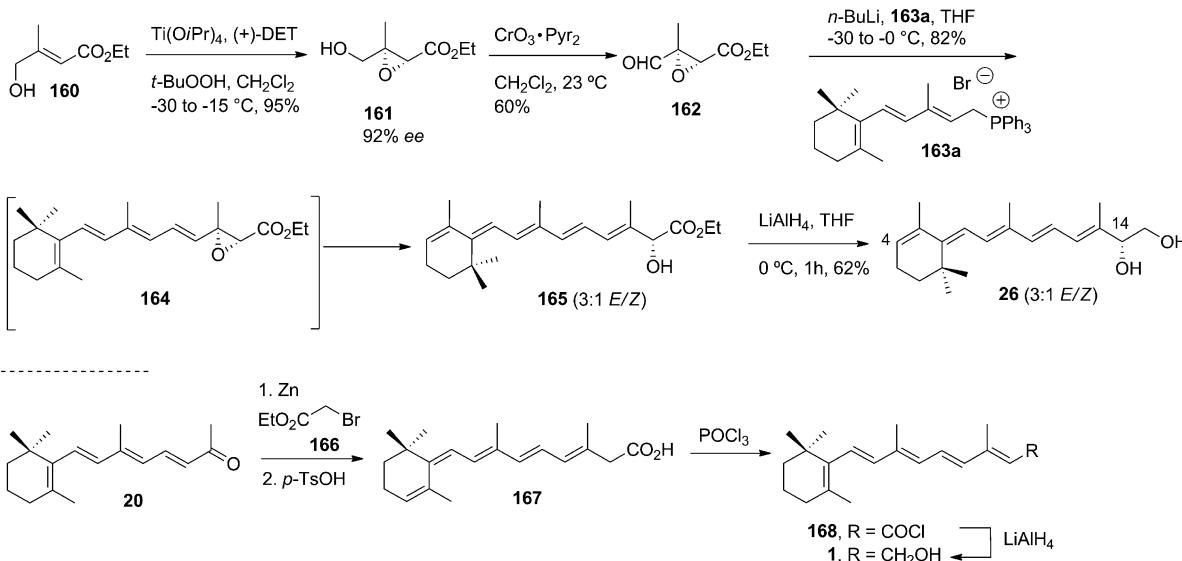
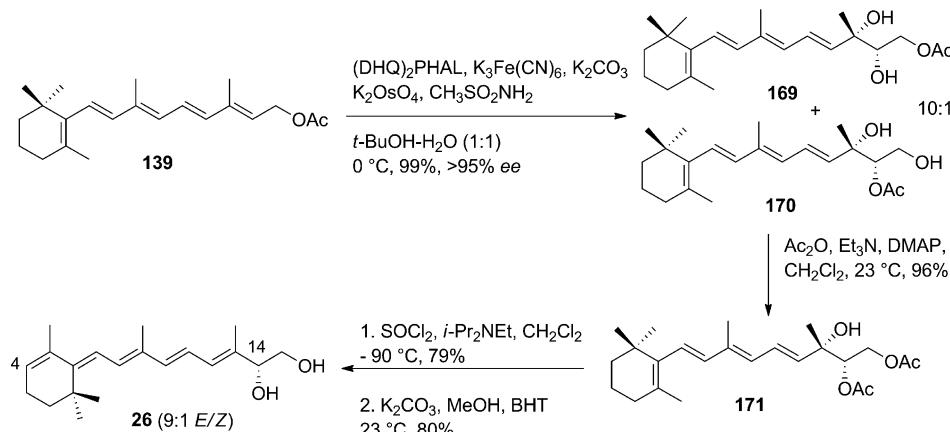
The approach to vitamin A (**1**) developed industrially at Roche started with the Darzens homologation of β -ionone (**17**) to unsaturated C₁₄ aldehyde **141**. Addition of the dianion of C₆ enynol **142** to **141** afforded propargylic alcohol **143**. Lindlar hydrogenation to the bis-allylic secondary alcohol followed by selective acetylation provided **144**. Dehydration to vitamin A acetate (**139**), isomerization of the mixture of isomers, and saponification led to **1** (Scheme 12).³⁰²

Dioxepin **145** can be considered as a masked C₆ unit and has been used for the synthesis of 13-*cis*-11,12-didehydroretinol (**147**).³¹⁷ Addition of the lithium acetylid derived from **145** to C₁₄ enal **141** afforded the propargylic alcohol **146**, which was

treated with Burgess reagent at 80 °C to give the conjugated trienye. Upon hydrolysis of the (2*H*)-1,4-dioxepin ring, the intermediate was transformed into the corresponding retinal analogue **147**, which was obtained as a mixture of Z/E isomers at the terminal C13=C14 bond in a 4:1 ratio (Scheme 12).³¹⁷

The mechanistically unrelated, low-valent titanium-induced reductive elimination of but-2-ene-1,4-diols,³¹⁸ which has been applied to the formation of the (E,E)-1,3-diene central unit of retinol and analogues, also started from propargylic alcohols (Scheme 13).³¹⁹ Addition of the Grignard reagent derived from ethynyl- β -ionol (**148**) to ethyl χ -oxysenecionate (**149**) afforded the diol **150** in 90% yield as a mixture of diastereoisomers. Reduction and protection of the primary alcohol could be followed either by *syn* semihydrogenation of **151** using Lindlarr catalyst or by *anti* reduction with LiAlH₄. Low-valent titanium, prepared by reacting a THF mixture of LiAlH₄ and TiCl₃ in a 1:2 ratio, effected the reductive elimination of either isomer of **152** at room temperature to provide stereoselectively the TBDMS-protected retinol **153** in 85% yield. Ethyl Z-oxysenecionate provided instead 13-*cis*-retinoic acid via the intermediate hydrogenation.³²⁰ The use of the corresponding C₅ dithioacetals in analogous sequence provided all-*trans*-retinal (**2**) after deprotection.³²¹

In some cases, the extended dehydration involves the secondary C4 allylic position and produces *retro*-retinoids. This reaction was used for the construction of the polyene side-chain of 14-HRR (**26**) from the condensation product of β -ionone (**10**) and alkynyl organometals derived from the “chiral pool” (Scheme 14). Starting with a derivative of (R)-glyceraldehyde acetonide as the source of chirality, protected enyne diol **156** (a separable 3:1 E/Z mixture) was formed by condensation of **154** with diethyl (3-trimethylsilyl-2-propynyl)-

Scheme 15. Synthesis of *retro*-Retinoids by Extended 1,10-DehydrationScheme 16. Synthesis of (*R*)-14-Hydroxy-4,14-retro-Retinol (26) Using the SAD and Extended 1,10-Elimination

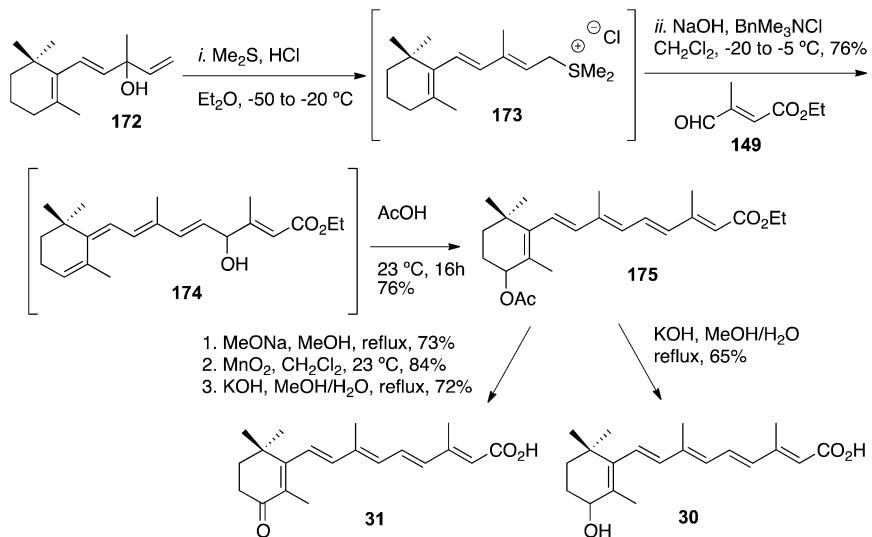
phosphonate (155) and two-step deprotection. A series of protective group transformations from the diol to the silyl-protected primary alcohol was required before the Mitsunobu inversion of the secondary alcohol could be effected. Deprotection of the benzoate and silyl ether groups followed by diol protection as disiloxane set the stage for the addition of the alkynyl anion derived from 158 to β -ionone (10) and reduction of the propargylic alcohol with Red-Al. Extended 1,6-dehydration of 159 using PPTS and final deprotection of the diol afforded a mixture of (*R*)-(+)14-HRR and its isomers (7:3 ratio) in 57% yield.³²² The (*S*) enantiomer was made similarly from intermediate 157.

8.1.4. Extended Eliminations. In the first approach to 14-HRR (26) reported (Scheme 15), the C₁₅ + C₅ condensation mode was followed for the reaction of phosphorane derived from phosphonium salt 163a and enantio-enriched epoxyaldehyde 162 (obtained by enantioselective Sharpless asymmetric epoxidation of alcohol 160 and oxidation of 161 with Collins reagent). The spontaneous ring-opening and double-bond shift on intermediate 164 (formally an extended 1,10-elimination process) provided a mixture of isomers of the *retro*-retinoid structure 165 in which the all-*trans* predominated.⁷⁵ A similar outcome was observed in the Reformatsky reaction of ethyl bromoacetate (166) and C₁₃ ketone 20 to give 4,14-retro-

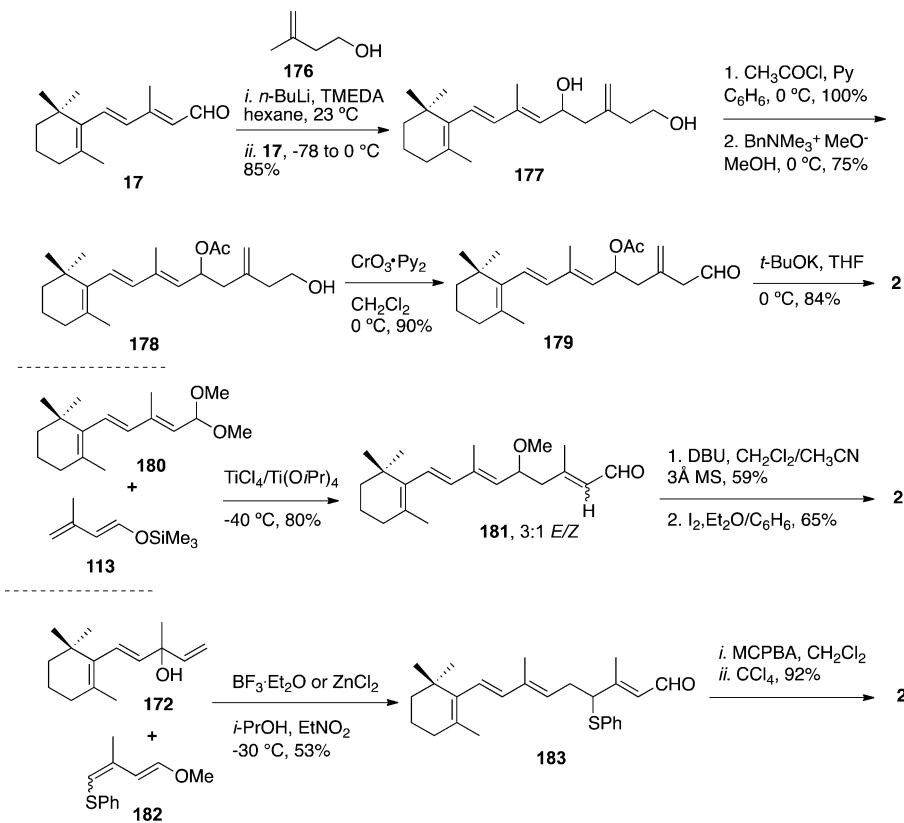
retinoic acid (167) by extended dehydration of the β -hydroxyester intermediate with *p*-TsOH. Treatment with POCl₃ returned the normal polyene by isomerization, and reduction of retinoyl chloride 168 with LiAlH₄ generated vitamin A (1) (Scheme 15).³²³

A short straightforward procedure for preparation of 14-HRR (26) was based on the direct Sharpless asymmetric dihydroxylation (SAD) of retinyl acetate 139.³²⁴ The highly enantioselective osmylation provided a 10:1 mixture of isomeric monoacetates of (13*S*,14*S*)-13,14-dihydroxyretinol showing acetate scrambling by acetyl O,O-migration (10:1 ratio of primary 169 to secondary acetate 170), which was converted into the diacetate 171, dehydrated by treatment with thionyl chloride (an extended 1,10-elimination of the chloride) to a 10:1 mixture of the *trans* and *cis* isomers, and saponified with potassium carbonate to give 26 (Scheme 16).³²⁴ Vitamin A acetate (139) is a convenient precursor of the corresponding 4,14-retinylacetate, which is generated by acid treatment (HBr, CH₂Cl₂, 30 s, 97%).³²⁵

A *retro*-retinoid structure 174 was formed as intermediate in the condensation of aldehyde 149 and the anion of sulfonium salt 173, most likely as a consequence of the extended 1,8-elimination of dimethyl sulfide formed.³²⁶ Upon addition of AcOH, the vinylogous aldol-like product 174 underwent

Scheme 17. Extended 1,8-Elimination with Formation and Trapping of a *retro-Retinoid* Structure

Scheme 18. Miscellaneous Methods for the Formation of All-trans-retinal (2) by Elimination Reactions



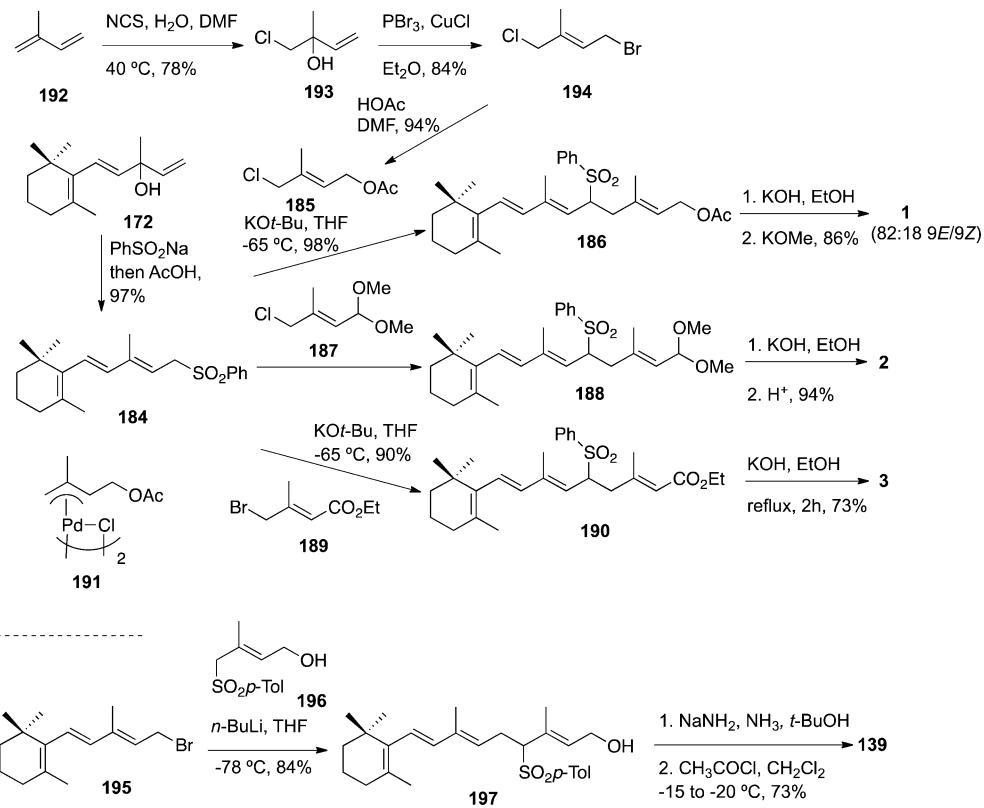
dehydration and capture of the conjugated carbenium ion by the solvent at the C4 position (Scheme 17). Saponification of 175 afforded all-*trans*-4-hydroxyretinoic acid (30), whereas the alternative sequence of acetate hydrolysis, oxidation of the alcohol with MnO₂, and saponification, generated all-*trans*-4-oxoretinoic acid (31) as major isomer.³²⁶

8.1.5. Other Methods of Carbonyl Addition/Elimination. After reaction with electrophiles, allyl organolithium compounds with five carbon atoms incorporate the C₅ isoprene unit to the retinoid chain. Thus, the addition to C₁₅ aldehyde 17 of the dianion obtained by metalation of 3-methylbut-3-en-1-ol (176) with *n*-BuLi and TMEDA produced diol 177

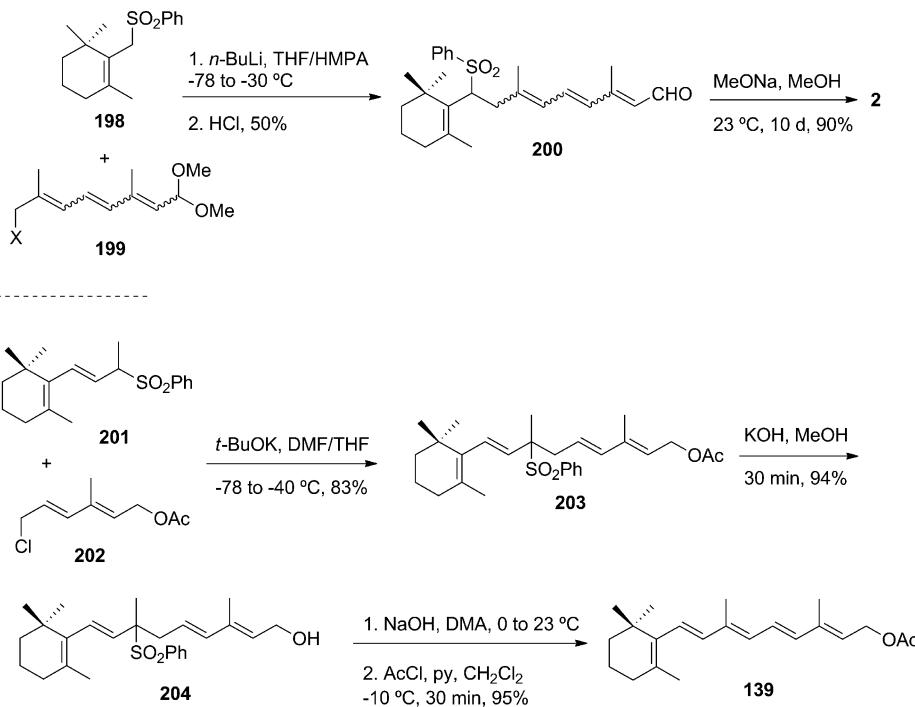
(Scheme 18).³²⁷ Diacetylation and selective saponification gave rise to alcohol 178, which was oxidized with Collins reagent to β,γ -unsaturated aldehyde 179. Concomitant isomerization to the α,β -unsaturated aldehyde and elimination of AcOH could be induced in this order by treatment of 179 with *t*-BuOK in THF to afford all-*trans*-retinal (2).

In classical Mukaiyama reactions, 1-trimethylsiloxybuta-1,3-dienes derived from senecialdehyde (such as 113) reacted with C₁₅ acetal 180 (which was obtained following the same sequence from β -cyclocitral 14) activated by ZnCl₂ or TiCl₄ in a regioselective γ -1,4-addition mode to afford the corresponding δ -alkoxy- α,β -unsaturated aldehydes 181 in high yield but as

Scheme 19. The (Marc) Julia Approach to the Formation of the C11–C12 Bond of Retinoids



Scheme 20. The (Marc) Julia Approach to the Formation of the C7–C8 and C9–C10 Bonds of Retinoids

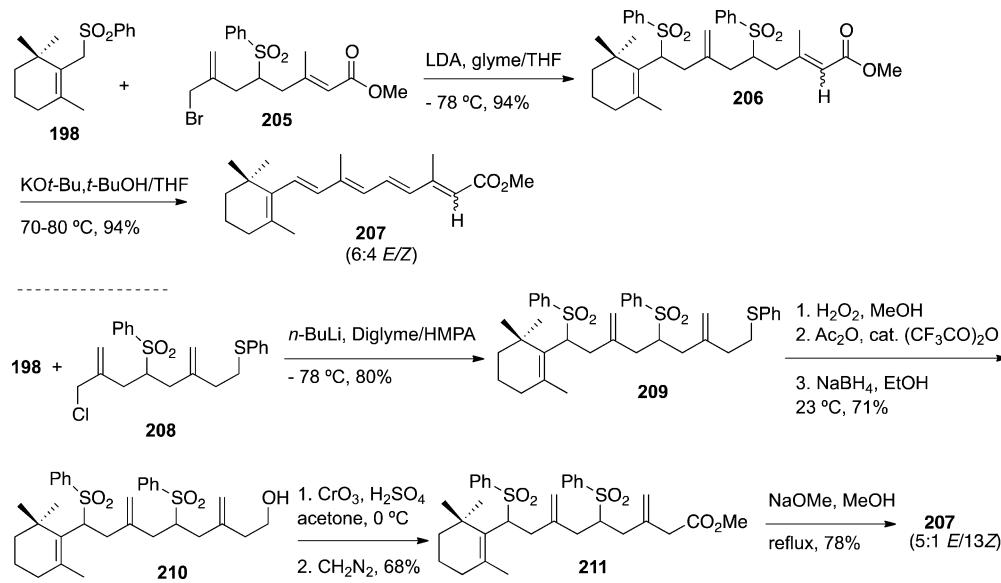


a mixture of *E/Z* isomers (Scheme 18). These intermediates were easily transformed into all-*trans*-retinal (**2**) by treatment with DBU and isomerization of the ca. 3:1 mixture of all-*trans*/13-*cis* isomers with catalytic amounts of iodine.³²⁸

When an allylic alcohol and a C₂-substituted (Br, SPh) enol ether are employed in a Mukaiyama reaction, the oxidation

state of the electrophilic reagent is decreased from carbonyl to alcohol, and the oxidation state of the nucleophilic component is increased, a so-called Oxidation State Modification (OSM) event. Activation of vinyl- β -ionol (**172**) with BF₃·OEt₂ or ZnCl₂ and 1 equiv of *i*-PrOH, followed by treatment with dienol ether **182** in nitroethane at -30 °C afforded aldehyde

Scheme 21. The (Marc) Julia Approach to the Formation of the C7–C8 Bond of the Retinoids by Double Elimination from Bis-sulfones



183. The oxidation of the sulfide with MCPBA provided all-*trans*-retinal (2) and other isomers in 92% yield after spontaneous elimination of the sulfoxide moiety (Scheme 18).³²⁹

8.1.6. Alkylation of Sulfones and Elimination. Marc Julia and Jean-Marc Paris discovered that carbanions stabilized by sulfones could be alkylated with alkyl halides, and the resulting alkylated sulfones, upon base-promoted elimination of phenylsulfenic acid, yielded olefins.³³⁰ The Julia condensation is the base of Rhône-Poulenc's (today Sanofi) industrial syntheses of vitamin A acetate.^{304,331} An extension of this process was the preparation of methyl retinoate³³¹ and vitamin A by the C₁₅ + C₅ route.³³² All synthesis based on sulfone elimination methods give in general moderate stereoselectivities, and variable amounts of *cis* isomers are obtained.

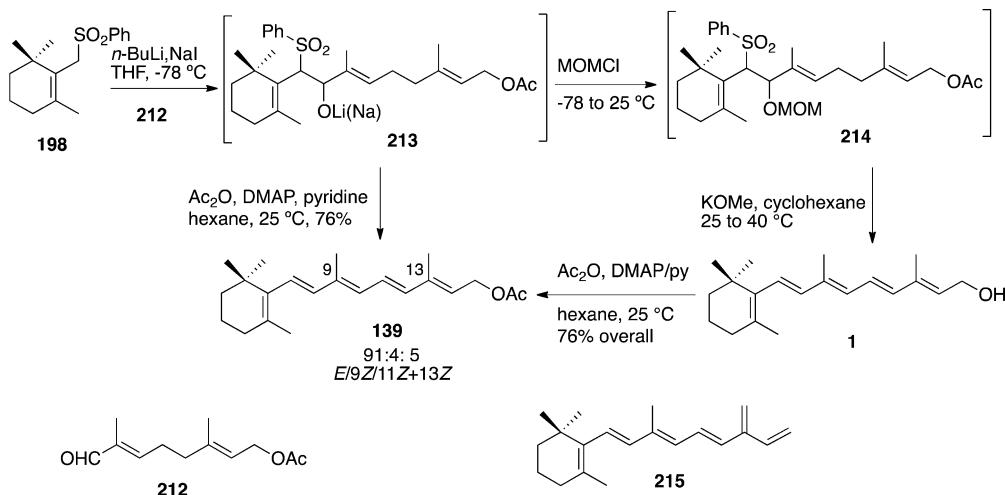
The route developed by Julia started from vinyl- β -ionol (172), which was treated with the phenylsulfinate anion to give the C₁₅ sulfone 184. Alkylation with 4-acetoxy-1-chloro-2-methylbut-2-ene 185 (KOt-Bu, THF, -65 °C, 98%) generated the C₂₀ acetate 186 (Scheme 19). Saponification of the acetate and elimination of phenylsulfenic acid in the heterogeneous phase afforded in 86% yield a mixture of all-*trans*- and 9-*cis*-retinol in an 82:18 ratio.^{304,331} A more recent version has optimized the process starting from isoprene (192). The halogen-selective substitution of (*E*)-1-bromo-4-chloro-3-methylbut-ene (194), prepared in two steps from 192, provided the corresponding C₅ unit 185 (other nucleophiles, such as alcohols, thiols, carboxylic acids, and amines, could be similarly used). The substitution of the allylchloride in 185 by the anion of C₁₅ sulfone 184 (*t*-BuOK, DMF, -20 °C, 14 h, 98%) led to adduct 186, which underwent base-induced β -elimination (NaOEt, EtOH, 94%) to give all-*trans*-retinol (1).³³³ The C₅ acetal 187 was selected instead for the preparation of all-*trans*-retinal (2).^{304,331} Retinoic acid and analogues modified at C13 have been synthesized by this method starting from ethyl γ -bromoseneconate (189) and derivatives.³³⁴ The dimeric π -allyl palladium complex 191 (generated from prenylacetate and PdCl₂) also functioned efficiently as electrophile in the sulfone alkylation reaction in a related approach to vitamin A.³³⁵

The positional exchange of the functionalities within the same condensation pattern was carried out starting from the alkylation of C₁₅ halide 195 and C₅-sulfone 196 to generate the C₂₀-sulfone 197 (Scheme 19).³³⁶ The treatment of 197 with sodium amide induced the elimination of phenylsulfenic acid; final acetylation produced vitamin A acetate (139).^{332b} This version, however, is limited by the instability of the allylic halide and was not developed commercially by Roche.

Alternative tactics for the construction of the retinoid skeleton by Julia reactions are shown in Scheme 20. The C₁₀ + C₁₀ condensation pattern involving an allyl chloride at the terminus of a protected trienal was used by Julia for the synthesis of retinal.³³⁷ The alkylation of β -cyclogeranyl sulfone 198 with allyl chloride 199 (an 80:20 mixture of isomers was used) and subsequent elimination of phenylsulfinate anion on 200 by treatment with MeONa in MeOH afforded, after 10 days, all-*trans*-retinal (2).³³⁷ The alternative C₁₃ + C₇ approach to vitamin A and its acetate (139) used the condensation of the anion of β -ionylsulfone (201) and chloride 202 to obtain sulfone 203. Saponification to 204 was followed by elimination of phenylsulfinate using NaOH in DMA. The process can be performed as a one-pot reaction by heating 201 and 202 with NaOH in DMA, but the content of the all-*trans*-retinol isomer did not exceed 30% using this variant (Scheme 20).³³⁶

Other versions of the Julia reaction have been based on double elimination processes from nonconjugated precursors. Addition of the anion of C₁₀ sulfone 198 to C₁₀ bromide 205 gave a mixture of diastereoisomers, which additionally showed the C13=C14 bond partly isomerized (Scheme 21). The two sulfinate groups of 206 were eliminated using KOtBu to obtain the methyl ester of all-*trans*-retinoic acid (207) and its 13-*cis* isomer in a 83:17 ratio.³³⁸ A series of masked isoprenoid units were developed to prevent the isomerization of the terminal α,β -unsaturated fragment. For example, the C₁₀ chloride 208 with a sulfone at the bis-homoallylic position and a terminal sulfide reacted with the anion of 198 to furnish compound 209. Oxidation of the sulfide and Pummerer rearrangement of the sulfoxide followed by saponification gave alcohol 210. Jones oxidation of 210 and methylation of the acid with diazomethane was followed by the double desulfonylation of 211

Scheme 22. The “Integrated Approach” to Retinoids Based on a Double Elimination Reaction



induced by treatment with MeONa, which yielded a mixture of methyl all-*trans*-retinoate (**207**) and its 13-*cis* isomer in a 60:40 ratio (Scheme 21).³³⁹

The most efficient version of this process uses the coupling of the sulfone-stabilized anion of **198** and the α,β -unsaturated aldehyde **212** in what is the first step of a classical Julia–Lythgoe reaction. In a departure from the normal outcome, when treated with a metal alkoxide the acetate or the THP acetal underwent the consecutive abstraction of the γ and δ allylic hydrogens (H11 and H12, respectively) from these intermediates with elimination of the acetoxy and tetrahydropyranoyloxy groups to furnish the retinoids. This double elimination method applied to β -acetoxysulfones obtained by the C₁₀ + C₁₀ approach provided the conjugated pentaene of methyl retinoate³⁴⁰ and vitamin A acetate (Scheme 22).³⁴¹ The stereoselectivity in the generation of the retinoid C7–C12 triene fragment in this one-pot reaction was very high, and only minor amounts of the 9Z (4%) and 11Z + 13Z (5%) isomers were isolated as side products together with the all-*E* (91%). Vitamin A (**1**) and its 13Z-isomer (available from the double-bond isomer of enal **212**) were obtained in good yield only when the double-elimination reaction was performed in a hydrocarbon solvent. The advantage of this practical method for polyene construction is that the desired skeleton and labile double-bond system are generated in a single elimination step, and mostly as *E* isomers. This methodology has been successfully applied to the one-pot synthesis of retinol acetate (**139**), in what has been termed an “integrated chemical process”.³⁴² The one-pot procedure starts with the preparation of the lithium salt of **198** with *n*-BuLi at -78 °C, in the presence of NaI, followed by addition of aldehyde **212** to give the adduct **213**, which was immediately trapped with MOMCl. The MOM acetal **214** underwent double elimination upon treatment with KOMe to give retinol (**1**), which was converted to the corresponding acetate **139** with Ac₂O, DMAP, and pyridine (Scheme 22). Addition of NaI prevented the formation of polymeric material derived from the dendralene byproduct **215**, and allowed the alkylation of the alkoxide with the cheaper MOMCl (Finkelstein conditions). The yield of vitamin A acetate (**139**) was determined by HPLC to be about 76%, which is higher than the 67% obtained in the corresponding stepwise procedure. In this way, by designing a multistep process in which the experimental conditions are

shared by all individual steps, an increase in the overall yield can be achieved, with clear economic and environmental implications.³⁴²

8.2. Connective Condensation of Carbonyl Compounds with Heteroatom-Stabilized Reagents

This group of double-bond forming reactions includes the condensation of phosphorus-, sulfur-, and silicon-stabilized reagents with carbonyl compounds, processes that takes place with positional selectivity and, in most cases, with high stereoselectivity.

The Wittig reaction³⁴³ has a prominent status in retinoid (and carotenoid) history, as it was adapted to the industrial synthesis of vitamin A (**1**) and all-*trans*-retinoic acid (**3**),^{303,344} and also of β,β -carotene (**4**),³⁴⁵ soon after its discovery.³⁴⁶ The rapid implementation of the Wittig methodology to industrial settings^{303b} was due to some distinctive advantages of this powerful synthetic method: the position-selectivity of the double bond formed, the alkaline or virtually neutral reaction conditions compatible with acid-sensitive functional groups, and the in situ generation of the ylide. A drawback, besides the sensitivity of phosphoranes to the steric hindrance of the carbonyl compounds, is the uncertainty in predicting the *E/Z* ratio for trisubstituted olefin formation.^{343b} In addition, laborious procedures for separation of the product(s) from phosphine oxide limit the utility of the method. Mechanistic investigations of this formal metathesis of double-bonded functional groups concur with the concerted formation and decomposition of oxaphosphetanes regardless of the nature of the ylide, at least for lithium-free reaction conditions.³⁴⁷

The control of the geometries of trisubstituted double bonds in retinoids (C9=C10 and C13=C14) using Wittig condensations is challenging as the reaction partners are ketones and substituted-allylic (semistabilized) phosphoranes. Moreover, the regioselective preparation of α -branched allylphosphoranes from the precursor halides (and alcohols) is complicated by competing elimination reactions. Nevertheless, Wittig and related condensations reliably yield *E*-isomers of disubstituted olefins (C7=C8 and C11=C12 bonds) of the retinoid side-chain, in particular the C7=C8 bond, due to the bulkiness of the trimethylcyclohexenyl fragment and the thermodynamic destabilization of the 7*Z* isomers.

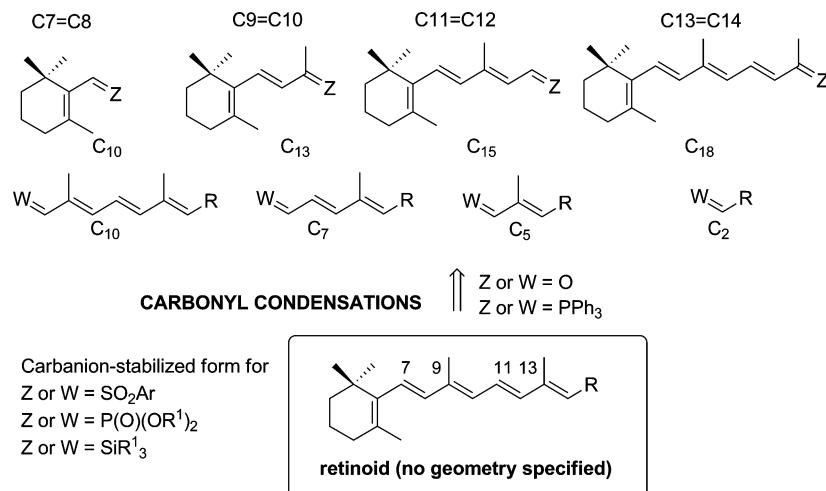
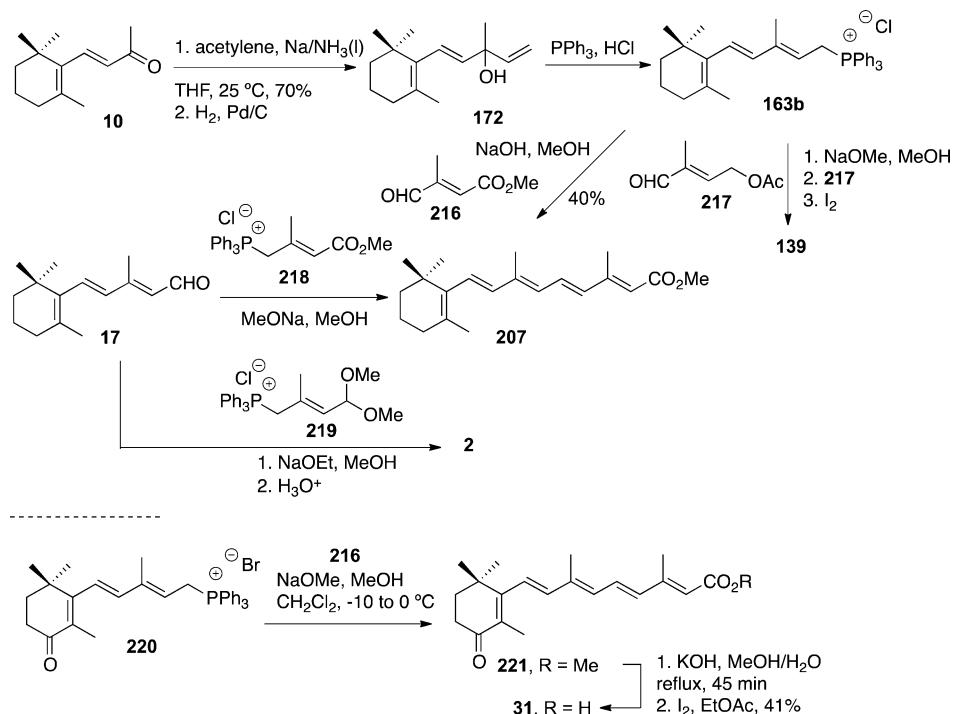


Figure 18. General construction of the retinoid side-chain by condensation of C_i and C_j ($i + j = 20$) fragments.

Scheme 23. The C11=C12 Retinoid Bond Formation by Wittig Reaction



The examples that follow have been selected to illustrate these principles and are organized according to the double bond of the retinoid skeleton formed in the key step, starting with the disconnection most often used (Figure 18).

8.2.1. Formation of the C11=C12 Bond. The formation of the disubstituted C11=C12 double bond has been the most prominent application of the Wittig and HWE reactions in this field, as the polyene chain is constructed by condensation of $C_{15} + C_5$ fragments, the latter being an isoprene derivative (Scheme 23).

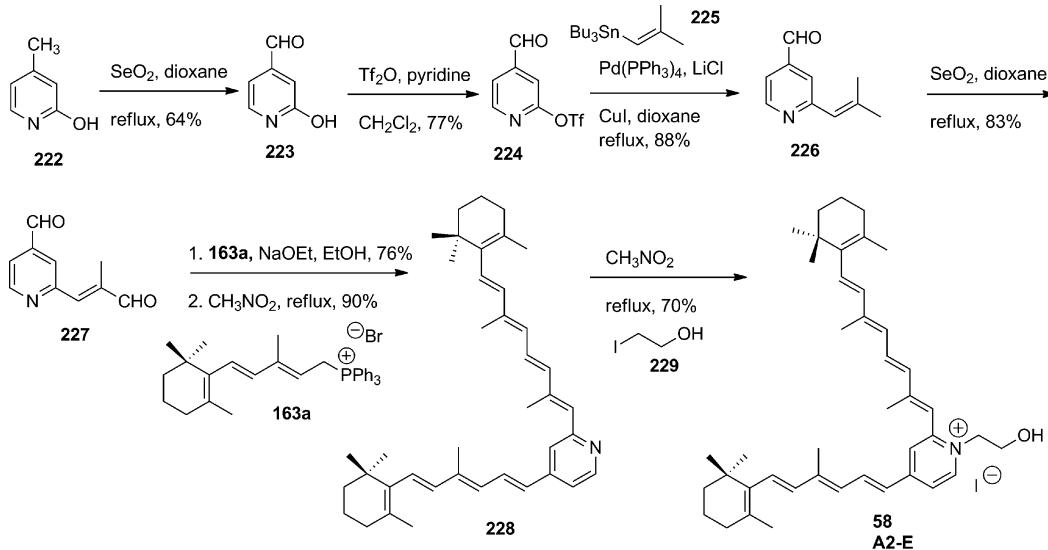
In the BASF industrial synthesis of vitamin A acetate (31), the treatment of the phosphorane derived from the phosphonium salt 163b (itself obtained from the rearrangement of allylic alcohol 172) with aldehyde 217 produced 139 as a mixture of isomers at the C11=C12 bond (Scheme 23). The ratio of isomers is solvent dependent, but the mixture was enriched for the *trans* isomer by iodine-induced isomer-

ization.^{303a} The use of 216 as partner of the phosphonium salt 163b directly led to methyl all-*trans*-retinoate (207).³⁴⁸ All-*trans*-4-oxo-retinoic acid (31) was prepared using the same approach, from the condensation of phosphonium salt 220 and aldehyde 216 under basic conditions (Scheme 23), followed by saponification and iodine-induced isomerization of the mixture of methyl 4-oxo-retinoate isomers 221 (41% combined yield).³²⁶ (*R*)-All-*trans*-3-hydroxyretinoic acid was made from similarly functionalized fragments.³⁴⁹

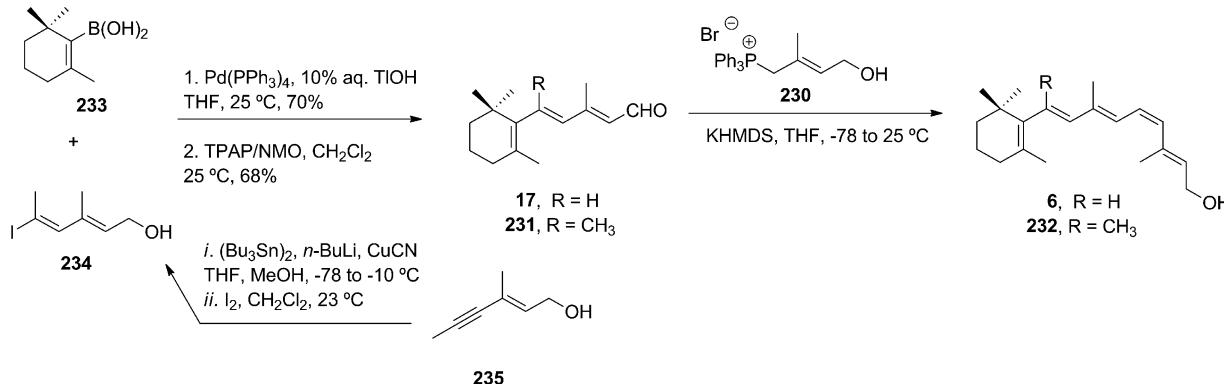
The exchange of functional groups in the condensation partners, using β -ionyldeneacetaldehyde 17 and phosphonium salts 219 and 218, led to alternative approaches to all-*trans*-retinal (2) and all-*trans*-retinoic acid (3), respectively, after acetal hydrolysis or saponification of 207 (Scheme 23).^{303a,350}

A double Wittig reaction of phosphonium salt 163a and bis-aldehyde 227 was used in the first reported synthesis of A2-E (58) (Scheme 24). The synthesis of the pyridine derivative 227

Scheme 24. The First Synthesis of A2-E (58) by Double Wittig Reaction



Scheme 25. Z-Selective Wittig Reaction in the Synthesis of 11-cis-Retinoids



started with the oxidation of 4-methyl-2-pyridone (**222**) to the corresponding 4-formylpyridone **223** with SeO_2 (64%), followed by formation of the triflate **224** with triflic anhydride in pyridine (77%), a CuCl/LiCl -accelerated Stille cross-coupling with **225** to give **226** (78%), and a second oxidation of the allylic position of **226** to produce **227** (83%). The Wittig reaction of **227** and the ylide derived from the treatment of **163a** with NaOEt in EtOH yielded a mixture of four isomers at the $\text{C}11=\text{C}12$ and $\text{C}11'=\text{C}12'$ bonds in a 4:3:3:2 ratio. Isomerization to a predominant trans product (90% yield) was induced by heating the mixture in nitromethane at 100 °C in the dark overnight. Alkylation of the pyridine bis-retinoid **228** with iodoethanol (**229**) yielded the fluorescent pigment A2-E (**58**).³⁵¹

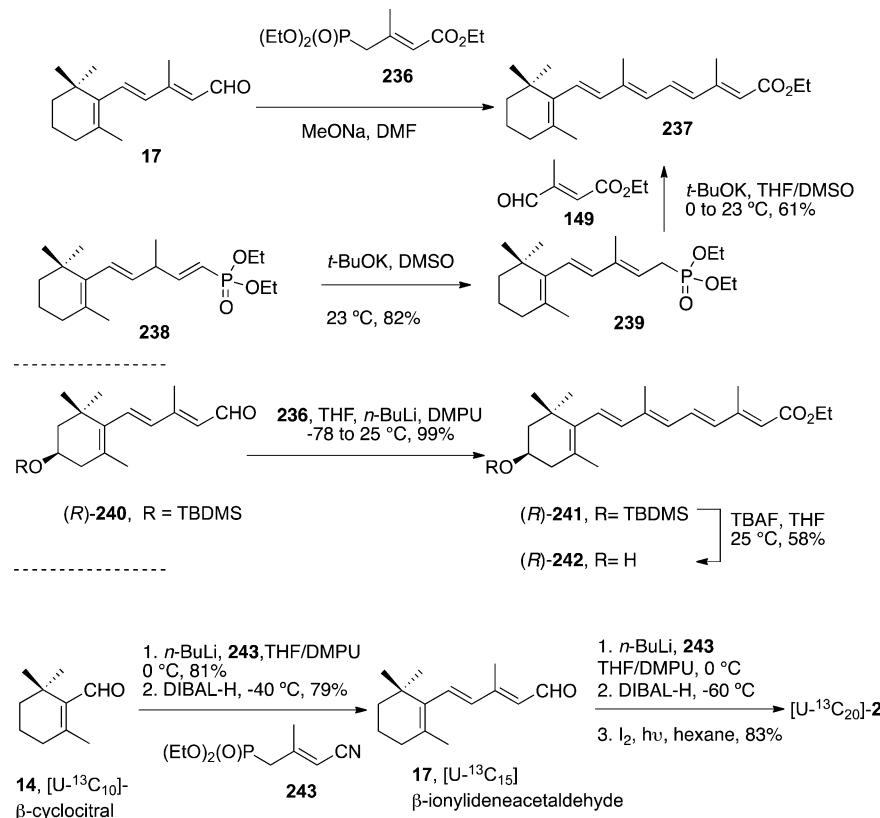
Whereas the $\text{C}_{15} + \text{C}_5$ approach to vitamin A using the Wittig reaction generates in general the *E* isomer at the newly formed $\text{C}11=\text{C}12$ bond, the dianions of hydroxyphosphonium salts (oxidoallylic phosphoranes) lead to *Z* olefins (Scheme 25). Treatment of the allylic phosphorane reagent derived from phosphonium salt **230** and 2 equiv of KHMDS in THF at -78 °C with **17** gave a mixture of 11-*cis*/all-*trans*-retinol isomers in a 83:17 ratio (40% yield).³⁵² This protocol was adapted to the preparation of the 7-methyl-substituted analogues **232** in similar yield and diastereoselectivity. Trienol **231** was obtained by Suzuki coupling of **233** and **234** using $\text{Pd}(\text{PPh}_3)_4$ as catalyst and a 10% aqueous TIOH solution,³⁵³ followed by oxidation

with catalytic quantities of tetra-*n*-propylammonium perruthenate (TPAP) and *N*-methylmorpholine *N*-oxide (NMO) as co-oxidant.³⁵⁴ 11-*cis*-7-Methylretinal (obtained by oxidation of **232**) has been used in a recent study of artificial visual pigments reconstitution between opsin mutants and synthetic chromophores with a restricted rotation at the C6–C7 bond.³⁵⁴

The first variants of the Wittig reaction were developed at BASF for the synthesis of intermediates toward the preparation of β,β -carotene (**4**), and used a dialkylphosphonate as an anion-stabilizing group.^{344,345b} Horner³⁵⁵ described the use of anions of phosphine oxides and phosphonates in Wittig-type reactions (Horner–Wittig reaction). In 1961, Wadsworth and Emmons described their work on phosphonate-stabilized carbanions and their condensation with carbonyl compound to afford olefins,³⁵⁶ and the process became known as the Horner–Wadsworth–Emmons (HWE) reaction.^{294a,357} Advantages of using phosphonate anions with an additional α -linked carbanion-stabilizing group are their increased reactivity relative to the traditional Wittig ylides derived from phosphonium salts, and the reversibility of the addition. This variant is suitable for the stereoselective preparation of trisubstituted unsaturated esters, nitriles, and ketones with high *E* stereoselectivity.³⁵⁶ Conditions have been developed, however, for the preparation of *Z* olefins, using modified phosphonate esters.^{298,299}

The HWE reaction was adapted at BASF for the preparation of all-*trans*-retinoic acid (**3**) by the $\text{C}_{15} + \text{C}_5$ condensation

Scheme 26. The HWE Reaction in the Synthesis of Retinoids by C11=C12 Bond Formation



scheme using **17** and **236** followed by the saponification of ethyl all-*trans*-retinoate (**237**) (Scheme 26).³⁵⁸ Allylphosphonate **239**, obtained by the isomerization of vinylphosphonate **238** induced by *t*-BuOK in DMSO, can be alternatively condensed with aldehyde **149** to stereoselectively yield **237**.³⁵⁹

A similar C₁₅ + C₅ approach was used in the synthesis of (R)-3-hydroxyretinoids.³⁶⁰ Pentaene (R)-**241** was obtained in excellent yield (99%) by HWE reaction between aldehyde (R)-**240** and phosphonate **236** using *n*-BuLi as base in THF and DMPU as cosolvent. Deprotection of (R)-**241** with TBAF in THF at 0 °C gave (R)-**242** in 58% yield (Scheme 26).³⁶⁰

Allylic phosphonate anions derived from C₅-phosphononitrile **243** have been very useful for the preparation of retinal analogues, including those labeled at selected positions. The HWE of labeled β-cyclocitral (**14**) with **243** followed by another C₅ chain extension furnished uniformly labeled ¹³C-retinal (**2**) (Scheme 26). Because the cyclohexenyl ring was prepared using ethyl acetoacetate, 4-bromo-2-methylbutene, and a C₂-phosphononitrile, the combination of these components with the labeled counterparts provided access to retinal with ¹³C isotopes at selected positions;^{122a} these probes enabled the study of retinal proteins with atomic resolution.^{122c}

A highly stereoselective synthesis of all-*trans*-retinoic acid (**3**) and of 9-*cis*-retinoic acid (**7**) used the HWE condensation of C₅-phosphonate **247** (as a 4:1 mixture of isomers) with the trienal iron tricarbonyl complex **246** and its isomer **252**, respectively (Scheme 27).³⁶¹ These compounds were made from the β-ionone-iron tricarbonyl complex **244** using alternative methods. Addition of the lithium anion of acetonitrile at -70 °C to **244** led to the (E)-nitrile **245** with the iron complexed to the terminal diene in 91% yield.³⁶¹ If the anion of ethyl acetate was used with **244** at the same

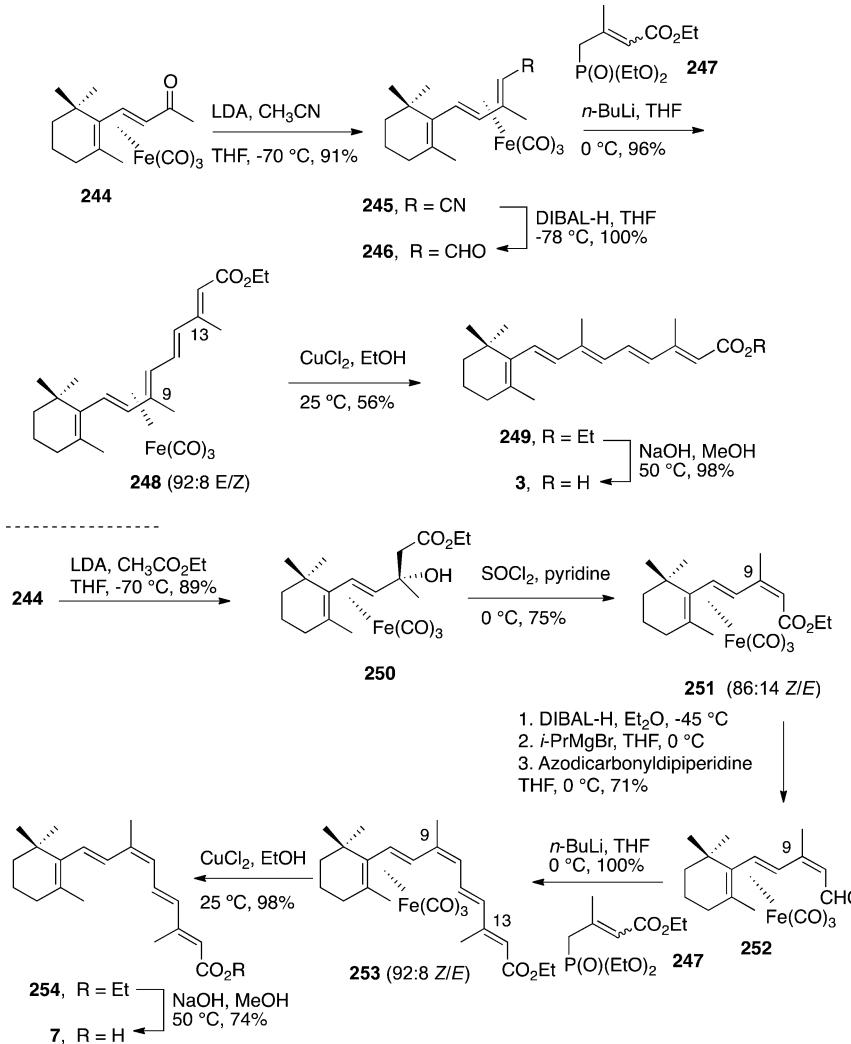
temperature, the major product after dehydration was the ester **251** of *cis* geometry, in which the metal is coordinated to the distal diene (65%). An *anti* elimination from a putative carbenium ion intermediate favored by chelation of the iron to the ester group would explain the geometry of product **251**. The oxidative decomplexation of **248** and **253** using CuCl₂ in EtOH and final saponification produced in high yields the carboxylic acids **3** and **7**, respectively.

8.2.2. Formation of the C13=C14 Bond. The formation of the terminal trisubstituted double bond of all-*trans*-retinoic acid (**3**) requires the condensation of C₁₃-ketone **20** (Scheme 2) and a two-atom partner. However, the reaction of **20** with triphenylphosphonium acetate led to ethyl retinoate in less than 15% yield.³⁰³ On the other hand, the HWE condensation of **20** with triethylphosphonoacetate (**256a**) or diethylphosphonoacetonitrile (**256b**) has been successful. The reaction of **20** with **256b** in the presence of MeONa provided retinoyl nitrile **121** in 80% yield. 4-Thioretinoic acid **257** was obtained in high yield as a 75:25 mixture of *E/Z* isomers, using the condensation of ketone **255** and phosphonate **256a** with NaH in DME, followed by saponification (Scheme 28).³⁶²

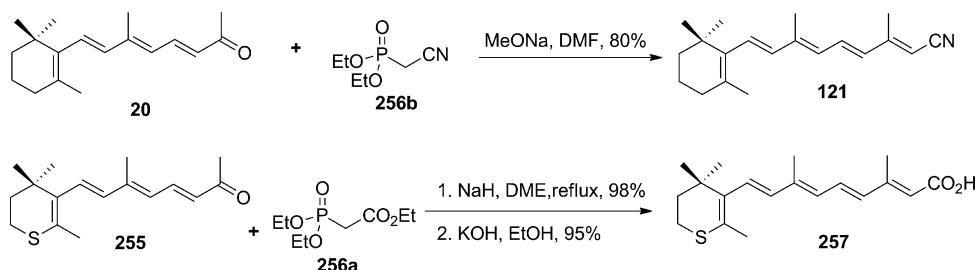
8.2.3. Formation of the C7=C8 Bond. The C₁₀ + C₁₀ scheme for the preparation of all-*trans*-retinoic acid (**3**) has been carried out through the Wittig condensation of the phosphorane derived from β-cyclogeranylphosphonium bromide (**258**) and unsaturated aldehyde **259** (Scheme 29) followed by hydrolysis.^{303a}

In contrast to the high *E*-selectivity of the C7=C8 bond formation using nonfluorinated trienals, the Wittig condensation of the lithium anion of phosphonium salt **258** and α-fluoro-substituted trienals afforded the corresponding 9-demethyl-9-fluororetinoates as a 4.5:1 mixture of isomers at

Scheme 27. HWE Reaction of Diastereomeric Trienal Irontricarbonyl Complexes To Afford Retinoic Acids 3 and 7



Scheme 28. The HWE Reaction in the Synthesis of Retinoids by C14=C15 Bond Formation



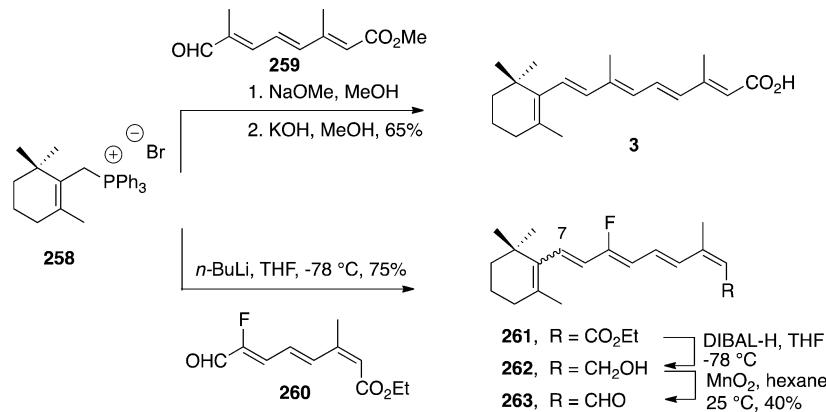
the C7=C8 bond.³⁶⁴ A similar result was obtained in the HWE condensation of the isomeric trienoate 260 during the synthesis of 13-cis-9-fluororetinol analogue 263 (Scheme 29).³⁶³

8.2.4. Formation of the C9=C10 Bond. The C₁₃ + C₇ pattern of retinoid construction is represented by the preparation of ethyl all-trans-retinoate (237) by condensation of the ylide generated from C₁₃ phosphonium salt 264 and aldehyde 265 (Scheme 30).^{303a}

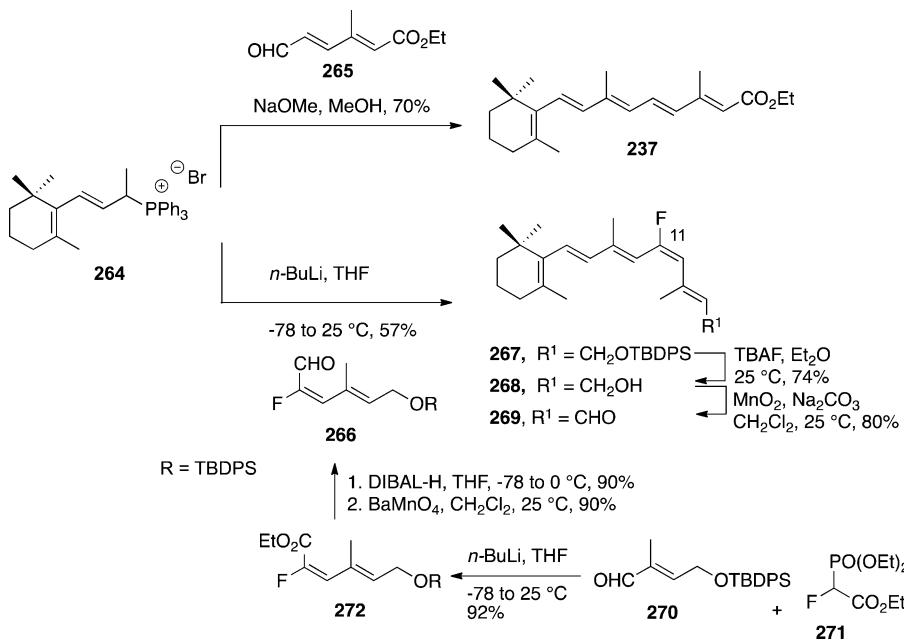
Unusual stereochemical outcomes have also been noted in the synthesis of some double bonds of the retinoid polyene chain substituted with fluorine atoms. The Wittig condensation of α -fluoro-unsaturated aldehyde 266 and the anion of allylic phosphonium salt 264 produced exclusively the trans-

trisubstituted olefin (cf., with H instead of F, a ca. 1.4:1 9E/9Z isomer ratio usually results), and led to the preparation of 11-cis-11-fluororetinoids 267–269.³⁶⁴ HWE condensations of fluorophosphonates are highly cis stereoselective (*E* geometric descriptor in the formed Csp²=Csp² bonds according to the CIP rules). Ethyl 2-(diethoxyphosphoryl)-2-fluoroacetate (271) reacted with aldehyde 270 to give 272 (Scheme 30), which is the reversal of the stereochemical outcome shown by the nonfluorinated HWE reagent. Retinal analogues with fluorine atoms at the even-^{121c} and odd-numbered positions of the side-chain³⁶⁴ serve as useful NMR probes for ligand–protein interaction studies.^{121c,365}

Scheme 29. The HWE Reaction in the Synthesis of Retinoids by C14=C15 Bond Formation



Scheme 30. The Wittig Reaction in the Synthesis of Retinoids by C9=C10 Bond Formation



The Julia–Lythgoe olefination comprises three steps: addition of the α -metalated phenyl sulfone to the carbonyl group, acylation of the β -alkoxysulfone (the two steps shown in Scheme 22), and reductive elimination with a single electron donor.³⁶⁶ Sylvestre Julia reported the simplified one-pot preparation of olefins using the spontaneous Smiles rearrangement of the intermediate β -hydroxybenzothiazol-2-yl sulfones formed by addition of the precursor (BT sulfones) to aldehydes, thus obviating the additional functionalization of the classical Julia method. Julia and Kocienski³⁶⁷ expanded the repertoire of heteroarylsulfones and contributed to place this condensation reaction at the forefront of the synthetic methodologies for double-bond formation.³⁶⁸ Some general stereochemical principles are emerging from the application of the Julia–Kocienski reaction to oligoene synthesis. Worthy of note is the Z-selective Julia–Kocienski olefination of allyl sulfones with unsaturated aldehydes.³⁶⁹ However, this version of the Julia–Kocienski olefination is of limited utility for the synthesis of 11-cis-retinoids (our unpublished results).

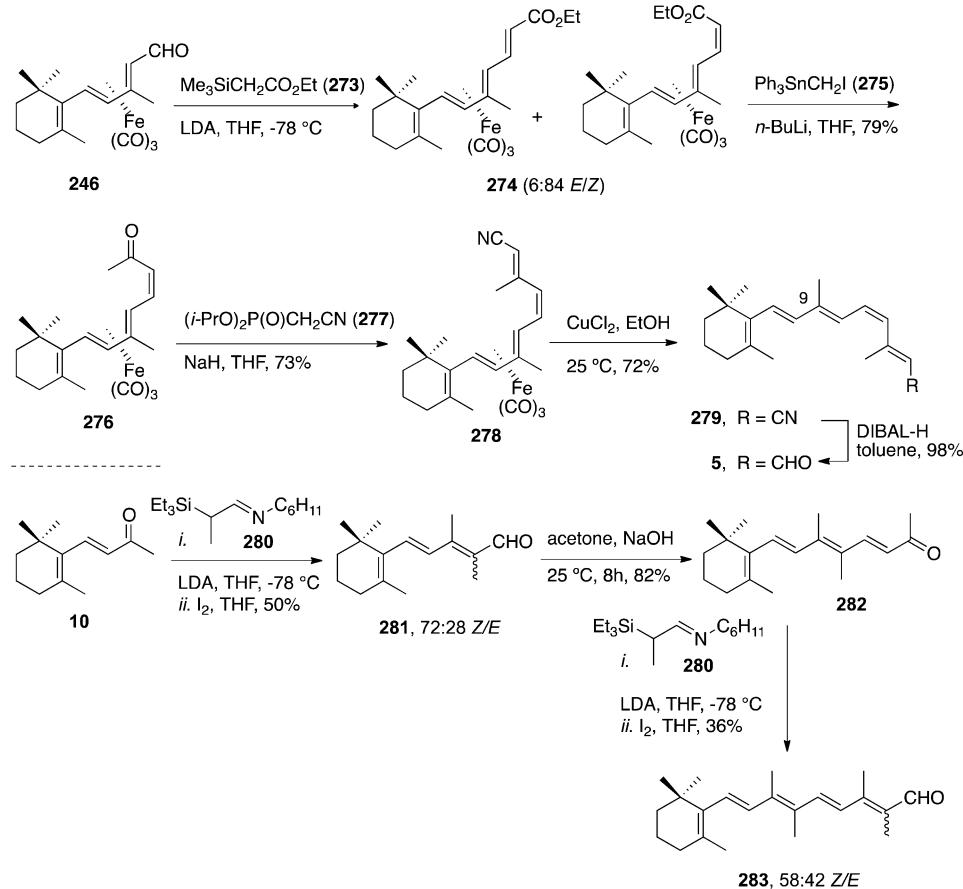
The Peterson olefination is in some settings advantageous over the Wittig reaction due to the greater reactivity and reduced sensitivity to steric hindrance of the silyl-stabilized

carbanions as compared to the phosphoranes. For simple alkenes, the choice of the elimination reaction conditions (acid or base) can provide stereocomplementary results.³⁷⁰

In the retinoid field, the Peterson olefination of diene–Fe(CO)₃ complexes has also yielded unanticipated stereochemical outcomes.³⁷¹ The condensation of 246 and the lithium derivative of ethyl trimethylsilylacetate (273) in THF at -70 °C afforded as major isomer compound 274 with a Z geometry in 77% yield (Scheme 31). This result stands in contrast to the stereoselectivity of the HWE reaction on the same substrate, which afforded the E isomer (Scheme 26). Ester 274 was converted in good yield (79%) into the C₁₈-ketone tricarbonyliron complex 276 using the lithium reagent derived from iodotriphenylstannylmethane (275). The HWE condensation of 276 with diisopropyl cyanomethylphosphonate (277) and NaH generated nitrile 278 as a single product in 73% yield. After decomplexation of 278 with CuCl₂, the resulting nitrile 279 was reduced to (11Z)-retinal (5) using DIBAL-H in toluene in excellent yield (98%).

The Corey–Schlessinger–Mills modification of the Peterson olefination³⁷³ has been used in the retinoid field for the synthesis of labeled retinals³⁷⁴ and analogues with tetrasub-

Scheme 31. The Peterson Reaction and Variants in the Synthesis of Retinoids



stituted double bonds, such as 10,14-dimethylretinal (**283**).³⁷² Reaction of β -ionone (**10**) with (α -triethylsilyl)-propionaldehyde cyclohexylimine (**280**) and LDA followed by treatment of the condensation product with iodine afforded **281** in 50% yield as a 72:28 mixture of *Z/E* isomers (Scheme 31). Aldol condensation of *E* trienal **281** with acetone gave tetraenone **282**. A second homologation of **282** with **280** provided with poor stereocontrol **283** as a 58:42 mixture of *Z/E* isomers at the terminal double bond.³⁷²

8.3. Pd-Catalyzed Cross-Coupling Reactions

Conceptually, the application of transition metal-catalyzed processes³⁰⁰ to retinoids complements the general condensation approaches described before, as it gives polyenes by single-bond construction between unsaturated carbons, instead of by double-bond formation. These procedures are in general mild and functional group tolerant, and the synthesis of the desired isomer can be achieved by choosing the geometries of the alkenyl reactants, because the oligoene products preserve in general the stereochemical information of the cross-coupling partners.²⁹⁶

Alkenyl intermediates and reagents are routinely prepared from alkynes by regio- and stereoselective hydrometalation, carbometalation, halometalation, and metallometalation.³⁷⁵ The *syn*-carbometalation, especially the Zr-catalyzed methylalumination of terminal alkynes, which can be followed by iododemetalation, has become a widely applicable tool for the synthesis of naturally occurring (*E*)-trisubstituted alkenes such as those present in retinoids and terpenes (Figure 19).³⁷⁶ Other methods include the palladium-catalyzed metalation of vinyl

halides, halogen–metal exchange/carbanion metalation of vinyl halides, and stereoselective reduction of alkynyl derivatives. Carbonyl compounds can alternatively be converted to one-carbon extended alkenyl organometals or alkenyl electrophiles by a number of condensation procedures, in particular the Takai–Utimoto reaction³⁷⁷ and the Stork–Bestmann variant of the Wittig olefination.³⁷⁸ All of these transformations being highly stereoselective, the entire collection of unsaturated organometallic reagents (B, Sn, Zn, Al, Zr) and electrophiles (bromides, iodides, triflates) with different geometries can be used in Pd-catalyzed cross-couplings, and most of the combinations have proven successful. Stille and Suzuki reactions using organostannanes and organoboranes, respectively, have been the most popular processes for retinoid bond construction. Comprehensive surveys of the application of Stille³⁷⁹ and Suzuki³⁸⁰ coupling to retinoids using complementary functionalized fragments for the formation of every side-chain single bond have been conducted.^{39a,381} Organoboron compounds can be prepared by hydroboration of alkynes or homologation of carbonyl groups, and tolerate a broader range of nontransferable functional groups incorporated into the organometallic reagent. The formation of alkenylorganostannanes usually starts from the corresponding alkynes, using copper or palladium-catalyzed hydro(alkyl)stannation.³⁸² The organoboron compounds generate nontoxic inorganic byproducts, which are readily removed by simple workup procedures. In general, the reactions are run in aqueous solutions with 2 equiv of an inorganic base.^{380,383} An additional advantage of organoboranes relative to organostannanes is their greater tolerance to steric hindrance on both coupling partners,

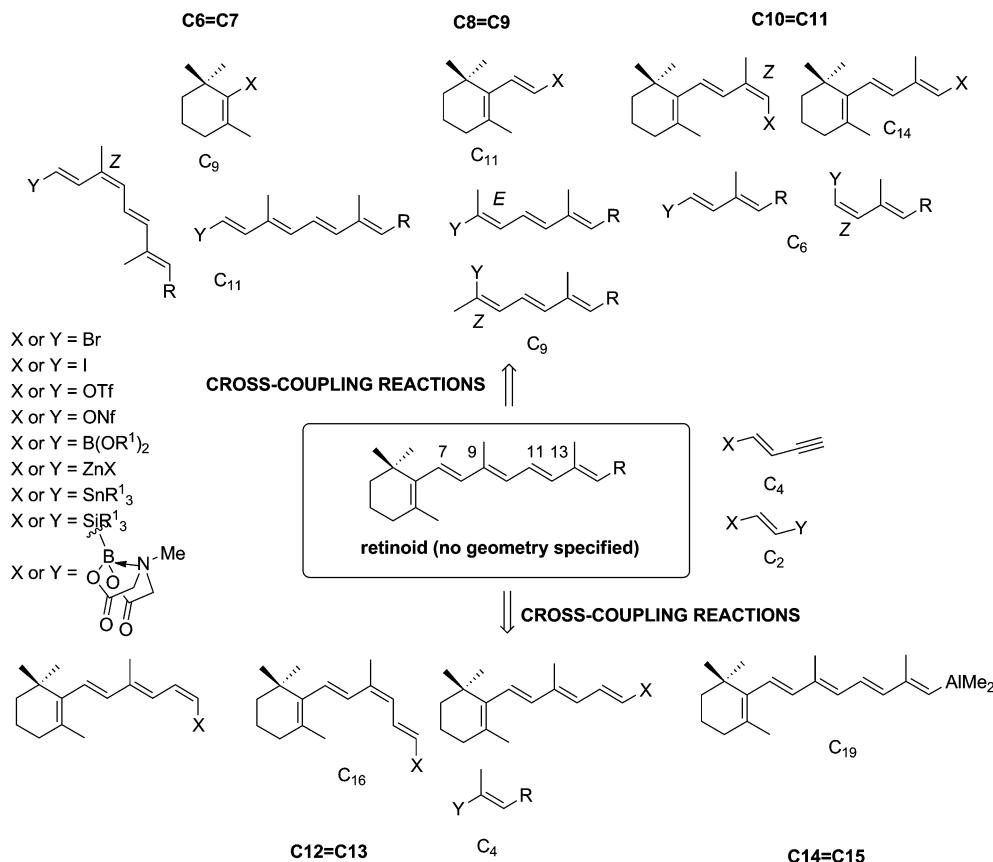
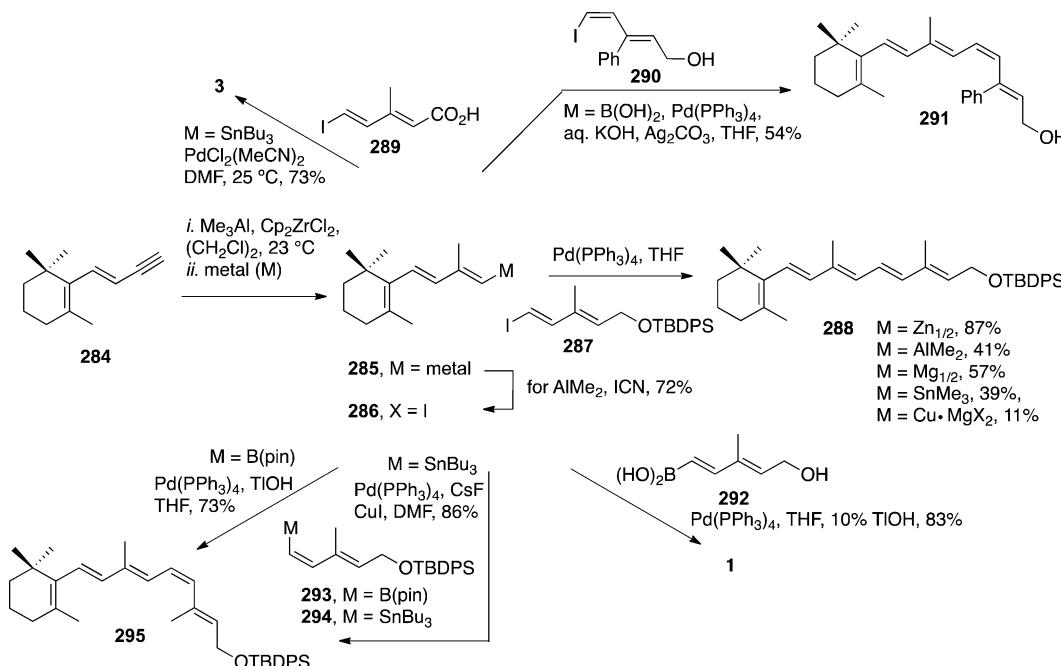


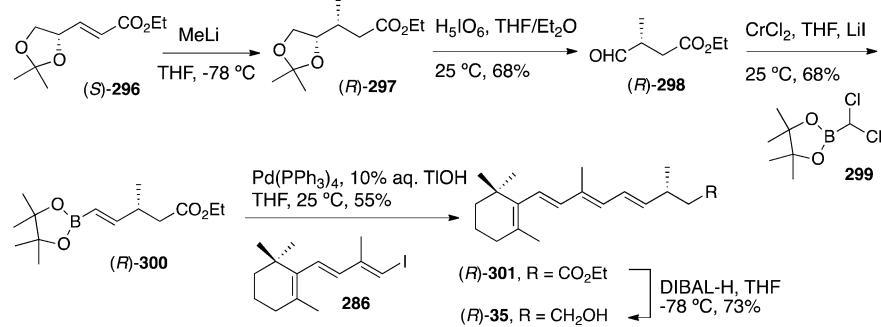
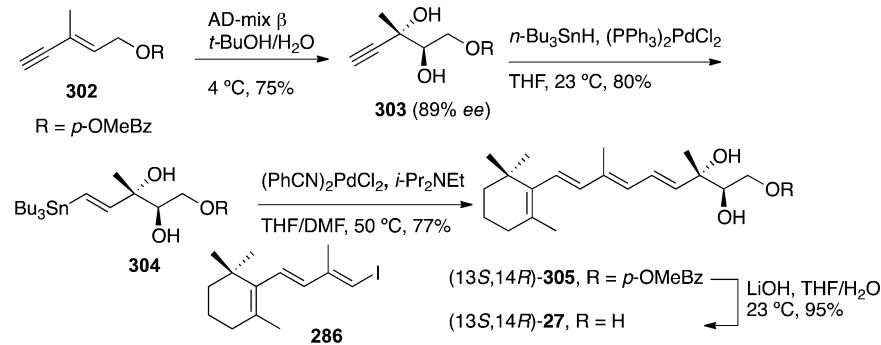
Figure 19. The building blocks for the synthesis of retinoids via $\text{Csp}^2\text{--Csp}^2$ bond formation.

Scheme 32. The $\text{C}_{14} + \text{C}_6$ Approach to Retinoids by C10–C11 Palladium-Catalyzed Bond Formation



which can be advantageous for the preparation of sterically hindered substrates.^{383,384} The Negishi coupling^{294b,375b} has been less frequently used, perhaps due to the sensitivity of the organozinc reagents to moisture and oxygen. More recent is the extension to retinoid synthesis of the Hiyama reaction³⁸⁵ using organosilanes.

Cross-coupling methods have a number of drawbacks,³⁸⁶ which are primarily related to the nature of the organometallic donors: (a) in Stille couplings, the homodimerization of the organotin reagents, which is partially solved by the use of excesses and forcing conditions, and the removal of the toxic tin byproducts; (b) in Suzuki couplings, the protodeborylation and

Scheme 33. The Stereoselective Synthesis of (*R*)-All-*trans*-13,14-dihydroretinol (*R*)-35 by C₁₄ + C₆ Suzuki ReactionScheme 34. The Stereoselective Synthesis of (13*S*,14*R*)-All-*trans*-13,14-dihydroxyretinol by C₁₄ + C₆ Stille Reaction

degradation during extended storage, which require that some boronic acids and esters must be synthesized and purified before the reaction. In addition, in certain cases of low reactivity, some erosion on the *E/Z* selectivity has been observed in these couplings.

8.3.1. Formation of the C10–C11 Bond. The C10–C11 bond formation benefits from the ready availability of the required fragments, C₁₄ alkenyl iodide 286 and C₆ organometals 292–294 or the reversed functionalities, 285 and 287, 289–290 (Scheme 32). C₁₄-functionalized compounds are made by zirconium-assisted carboalumination of alkyne 284 (the synthesis of which from β -ionone **10** is well-established)³⁷⁶ followed by transmetalation of the alkenylalane intermediate to give organometals 285 or iodination to produce 286.³⁸⁷ The C₆-functionalized dienyl iodides 287 and 289 of trans geometry can be prepared by hydrometalation of an enyne and iododemetalation.

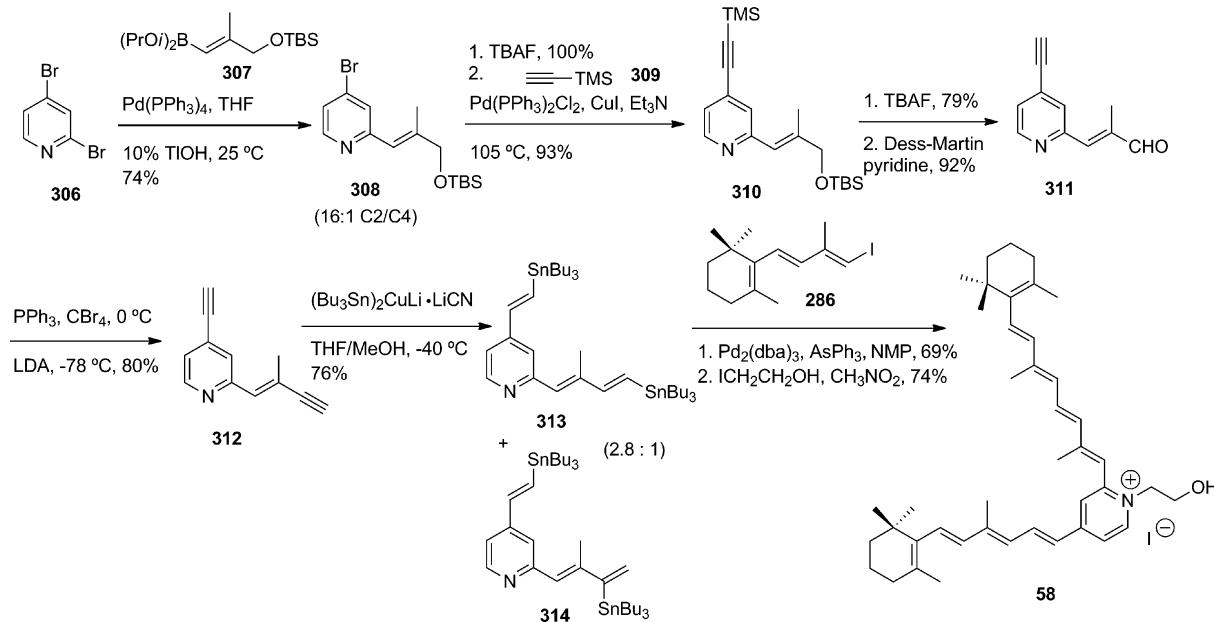
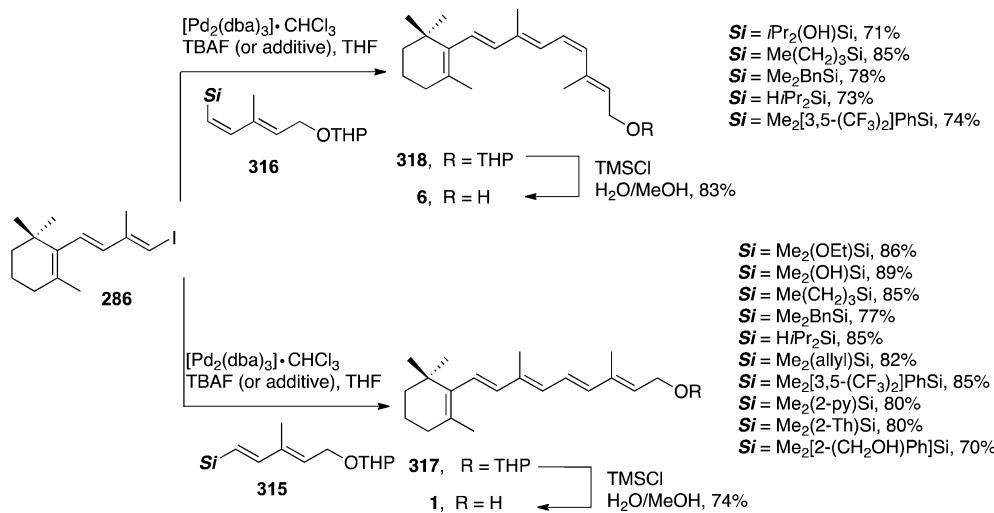
In the first application of this method to the synthesis of silyl-protected vitamin A 288 using a C₁₄ + C₆ approach, the Pd(PPh₃)₄-catalyzed cross-coupling of trienylmetal derivatives and dienyl iodides worked best with C₁₄-alkenyl diorganozinc reagents (285, M = Zn_{1/2}, Scheme 32).³⁸⁷ Catecholboronates and zirconium derivatives gave low yields of the protected retinol 288 (Scheme 32). Using the acceleration effect of thallium salts,³⁵³ natural and unnatural retinoids were prepared in moderate to good yields by the coupling of alkenyl iodides and alkenylboronic acids under mild conditions.³⁸⁸ For example, all-*trans*-retinol (**1**) was synthesized by the cross-coupling of dienylboronic acid 292 and trienyl iodide 286 with Pd(PPh₃)₄ and 10% aqueous TIOH at room temperature. Polyenes with sensitive *cis* double bonds such as the 11-*cis*-retinol analogues were similarly prepared because the mild reaction conditions preserved the *cis* geometry of the starting dienyl iodide (Scheme 32).^{388a} A phosphine-free version of the Stille reaction was used in the coupling of trienylstannane 285

(M = SnBu₃) and dienyl iodide 289 to afford all-*trans*-retinoic acid (**3**) in 73% yield.³⁸⁹ The functionalities of the coupling partners can be exchanged, as demonstrated by the Suzuki reaction of 285 (M = B(OH)₂) and *cis*-dienyl iodide 290, prepared by diimide hydrogen transfer reaction from the precursor alkynyl iodide, which provided the 11-*cis*-retinol analogues 291 with a phenyl group at the C13 position (Scheme 32).³⁹⁰

cis-Dienyl organometallic reagents 293 and 294 (pinacol boronates/tributylstannanes) are also useful components for this late-stage polyene construction tactic (Scheme 32).³⁹¹ These reagents were prepared using standard procedures from either enynes by hydrozirconation/protodemetalation of enynylboronates or enynylstannanes, or from the corresponding *cis*-dienyl iodide by halogen-metal exchange and trapping with triisopropylborate/pinacol or by Pd-mediated transmetalation. Both *cis*-dienyl organometals 293 and 294 underwent efficient Pd-catalyzed cross-coupling with trienyl iodide 286 (or trienyl triflate, not shown) under appropriate conditions to produce protected 11-*cis*-retinol 295.³⁹¹

The enantiomers of 13,14-dihydroretinoic acid were made using a Suzuki coupling as the connective method to form the C10–C11 bond (Scheme 33).^{90b} The addition of MeLi to ethyl (4*R*)- or (4*S*)-4,5-(*O*-isopropylidene)pent-2-enoate enantiomers (**296**) gave a mixture of the 1,4- and 1,2/1,4-addition products in 70% yield, where the relative configuration of the alkoxy and methyl groups in the 1,4 product **297** was *syn*. Ester (*R*)-297, obtained from (*S*)-296, was treated with peryodic acid to effect deprotection of the isopropylidene acetal and concomitantly induce the oxidative cleavage of the glycol to afford aldehyde (*R*)-298 (Scheme 33). The preparation of the alkenyl pinacol boronate (*R*)-300 involved the treatment of (*R*)-298 with the organochromium intermediate generated from dichloromethyl boron(pinacolate) 299 and CrCl₂ in the presence of LiI, according to the modified Takai procedure.^{377b}

Scheme 35. The Stereoselective Synthesis of A2-E (58) by a Two-fold Stille Reaction

Scheme 36. The Stereoselective Synthesis of All-trans- and 11-cis-Retinol by C₁₄ + C₆ Hiyama Reaction

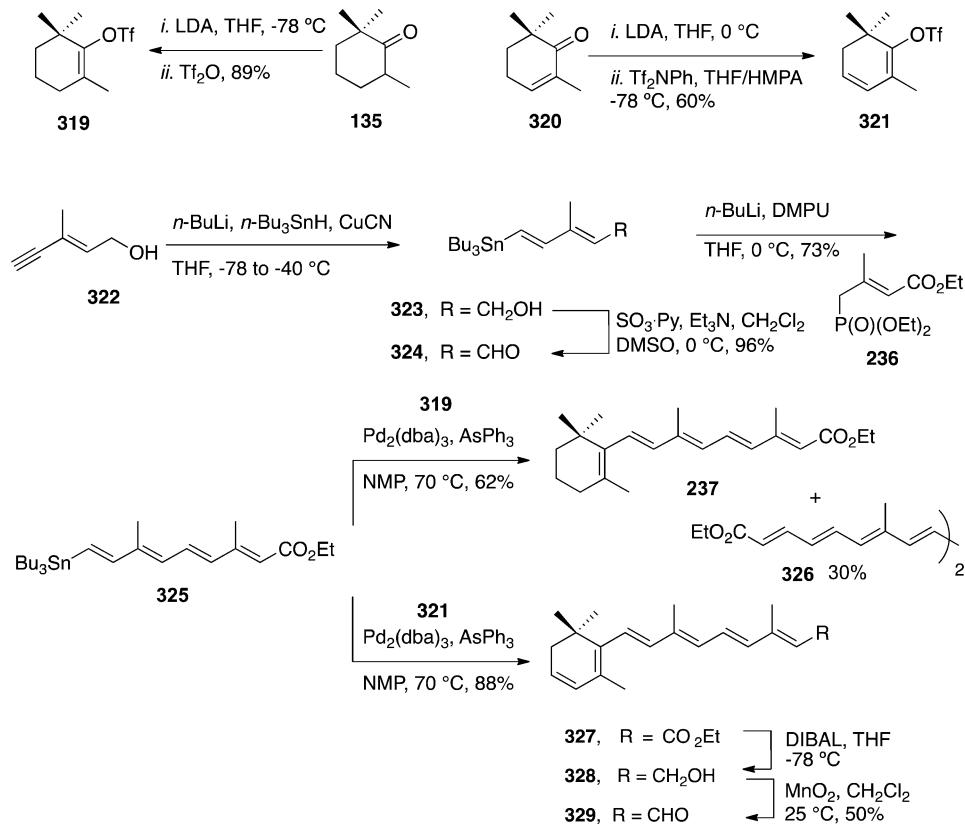
The Suzuki coupling of (*R*)-300 with trienyl iodide 286 gave tetraene ester (*R*)-301. Last, reduction of ester (*R*)-301 with DIBAL-H provided (*R*)-13,14-dihydroretinol (*R*)-35. Analogous sequence furnished (*S*)-35 from (*R*)-296.^{90b}

In a similar sequence, trienyl iodide 286 was coupled to stereodefined alkenylstannanes to access the four stereoisomers of 13,14-dihydroxyretinol (Scheme 34).³⁹² The synthetic scheme is only shown for the (13*S*,14*R*) stereoisomer of 27.³⁹² Sharpless asymmetric dihydroxylation (SAD) of the *p*-methoxybenzoates 302 (prepared in high yields by stirring *E*-enynol with *p*-anisoyl chloride and Et₃N in CH₂Cl₂ at room temperature) with AD-mix β under standard reaction conditions (*t*-BuOH/H₂O, 4 °C, 48 h) afforded protected triol 303. The palladium-catalyzed hydrostannation [*n*-Bu₃SnH, (PPh₃)₂PdCl₂, THF, 23 °C] produced in good yield alkenylstannane 304, which was then coupled to trienyl iodide 286 at 50 °C using (PhCN)₂PdCl₂ in the presence of Hünig base in DMF/THF. *p*-Methoxybenzyl ester (13*S*,14*R*)-305 was

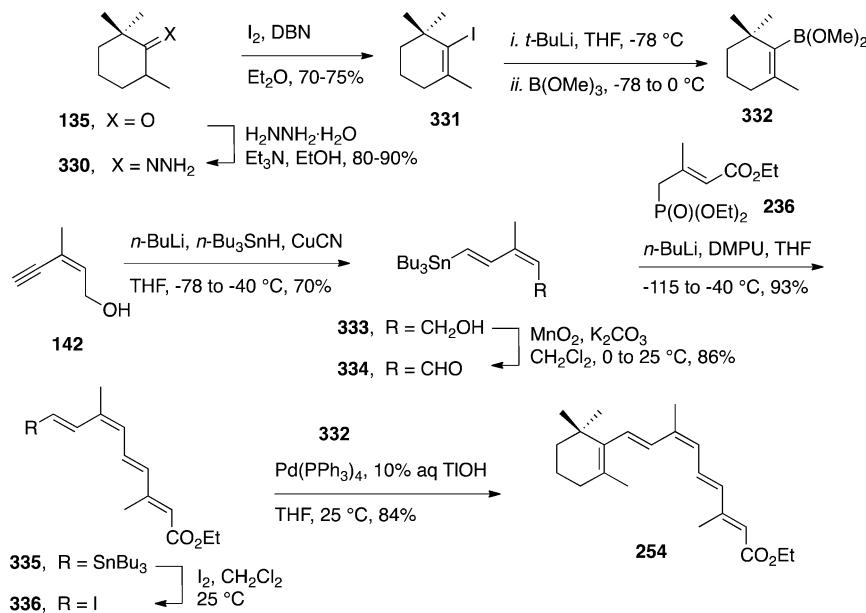
saponified with LiOH in THF/H₂O to provide (13*S*,14*R*)-27.³⁹²

An alternative approach to the A2-E fluorophore (58) (Scheme 35) was based on a double Stille cross-coupling of bis-stannane 314 and trienyl iodide 286 under Farina's conditions,³⁹³ which use "ligandless" Pd in combination with ligands of low donor ability in a polar aprotic solvent [Pd₂(dba)₃, AsPh₃, *N*-methylpyrrolidinone]. Other Pd-catalyzed cross-coupling was also used in the sequence employing various organometallic reagents (boron or zinc for the highly position-selective Suzuki or Negishi cross-coupling of 2,4-dibromopyridine 306; alkynyl copper for the Sonogashira coupling at the C4 position of 308). The copper-mediated *syn*-hydrostannylation of bis alkyne 312 was regioselective for the arylalkyne but not for the enyne, and afforded a 2.8:1 mixture of the terminal and internal dienylstannanes 314 and 313, respectively.³⁹⁴

The Hiyama cross-coupling has been praised as a cost-effective protocol as it uses organosilicon reagents that are

Scheme 37. The Stereoselective Synthesis of Retinoids by C₁₁ + C₉ Cross-Coupling Reactions

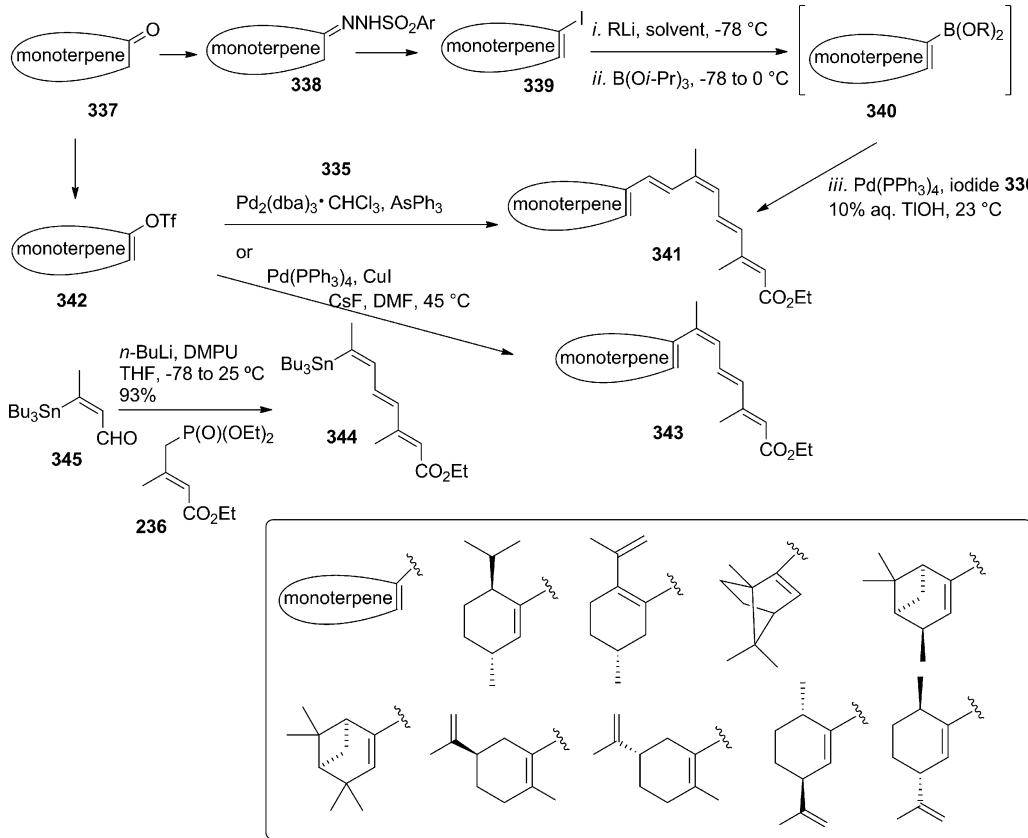
Scheme 38. A Suzuki-Based Synthesis of 9-cis-Retinoids by C6–C7 Bond Formation



easier to prepare and handle than other organometals, and are moreover more stable and less toxic than stannanes.^{385,395} Because a wide variety of heteroatom-functionalized silicon moieties, including halosilanes, oxysilanes, silanols, cyclic silyl ethers, polysiloxanes, and “safety-catch” silanols (which allows convenient choices of preparation and activation protocols) have been shown to couple efficiently under mild reaction conditions, the Hiyama coupling approach promises to find increasing applications in retinoid and polyene synthesis.³⁹⁶

The scope of the Hiyama reaction in the synthesis of all-*trans*- and 11-*cis*-retinoids and analogues using the highly convergent C₁₄ + C₆ strategy has been examined.³⁹⁶ To compare the performance of different silicon-based reagents, representatives of two families of oxygen-activated silanes and of the whole collection of “masked silanols” reported were prepared as C₆ *trans*- and *cis*-dienyl organometallic partners: oxygen-activated silanes (ethoxysilane and silanol) and “safety-catch” silanols (siletane, benzylsilane, silyl hydride, allylsilane,

Scheme 39. Synthesis of Terpene–Retinoid Hybrid Compounds by C6–C7 Bond Formation Using Cross-Coupling Reactions



phenylsilane, bis-trifluoromethylphenylsilane, pyridylsilane, thiophenylsilane, and “reusable” [2-(hydroxymethyl)phenyl]silane). These *E* and *Z* dienylorganometallic reagents 315 and 316 were prepared from the alkynyl derivatives according to standard procedures.^{396b} Yields of coupled products 317 and 318, respectively, were in general high regardless of the silicon reagent, with some exceptions (Scheme 36). Moreover, an integrated one-pot process was developed, which involves the Pt-catalyzed hydrosilylation of the precursor enyne with (HMe₂Si)₂O, and Pd-catalyzed cross-coupling with 286 to produce THP-protected all-*trans*-retinol (317) in 74% yield (Scheme 36).^{396b}

8.3.2. Formation of the C6–C7 Bond. The C6–C7 bond construction can be carried out by the coupling of functionalized C₉ cyclohexene and C₁₁ tetraene fragments. The reliable conversion of cyclohexanones to cyclohexenyl triflates by deprotonation/trapping with Tf₂O or PhNTf₂³⁹⁷ (Scheme 37), and to cyclohexenyl iodides by either oxidation of hydrazones³⁹⁸ or Shapiro reaction/trapping with iodine³⁹⁹ (see Scheme 38), provides convenient access to C₉ reagents. C₁₁-functionalized tetraenylstannane 325 was stereoselectively prepared in good yield by the HWE condensation of phosphonate 236 and stannyldienal 324. Stille coupling of 325 and 319 required heating to 70 °C due to the low reactivity of these hindered alkenyl triflates and gave 237 in 62% yield. Octaenedioate 326, the homodimer derived from stannane 325, was also obtained in 30% yield.³⁹⁷

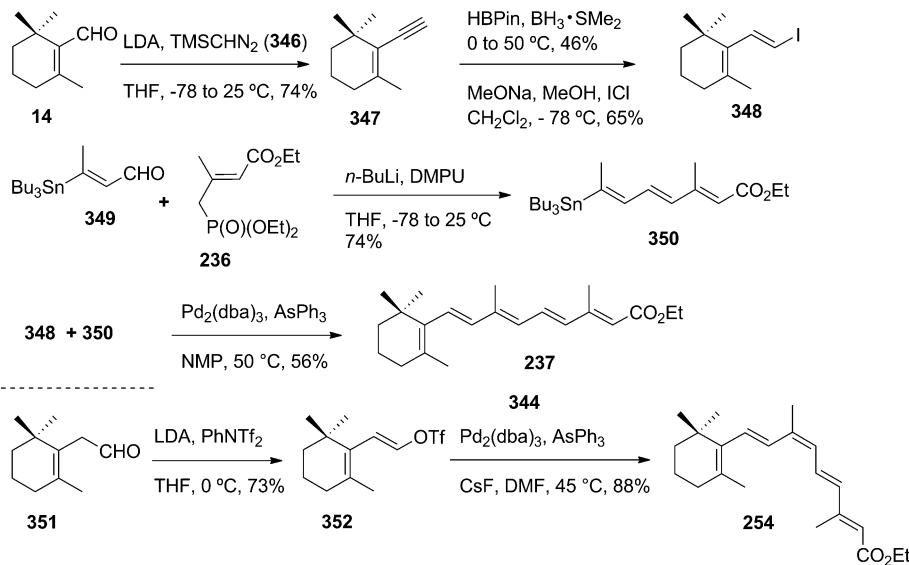
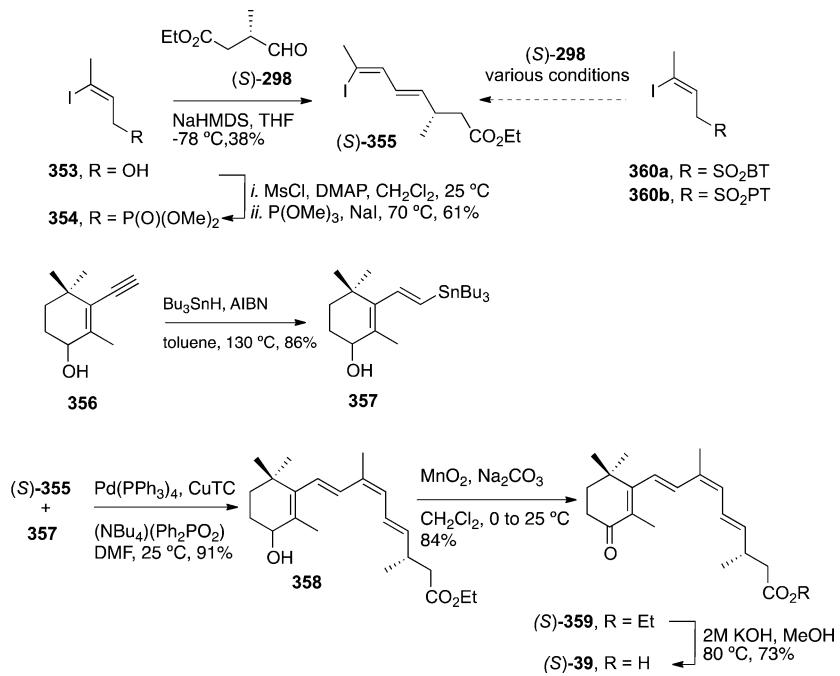
A synthesis of 3,4-didehydroretinoids (vitamin A₂ derivatives) with hexaene structure also followed this bond construction pattern (Scheme 37).⁴⁰⁰ Dienyl triflate 321 was prepared by deprotonation of 2,6,6-trimethylcyclohex-2-en-1-

one (320) with LDA, and trapping of the lithium dienolate with *N*-phenyltriflimide. The Stille cross-coupling using Farina’s conditions³⁹³ provided the hexaenyl ester 327 in 88% yield. Ethyl all-*trans*-3,4-didehydroretinol (328) was uneventfully reduced to all-*trans*-3,4-didehydroretinol (329) with DIBAL-H and the latter oxidized with MnO₂ to all-*trans*-3,4-didehydroretinol (329) in 50% combined yield.⁴⁰⁰

The dimerization of the tetraenylstannanes to 326 (Scheme 37) at the reaction temperatures (50–70 °C) required for the coupling of these hindered triflates using Farina’s conditions limits the application of the Stille reaction to cyclohexenyl triflates.³⁹⁷ This approach appears to be incompatible with the use of reagents with more sensitive *cis*-geometries of the side-chain. To overcome this shortcoming, the Suzuki cross-coupling, which is less sensitive to steric hindrance, was used in the same setting. In the synthesis of ethyl 9-*cis*-retinoate (254), cycloalkenylboronate 332 and *cis*-tetraenyliodide 336 were efficiently coupled after being generated *in situ* from the corresponding cyclohexenyl iodide 331 and tetraenylstannane 335, respectively.⁴⁰¹ Stannylcupration of enynol 142 to form dienylstannane 333 defines the *Z* geometry of tetraenyl iodide 336, because the HWE of 334 with 236 is *E* selective and the iodostannylation of 335 is stereoretentive (Scheme 38). The same synthetic strategy can also been applied to the preparation of ring-demethylated analogues as well as to other mono-*cis* (7*Z*) isomers of native retinoids.⁴⁰²

In an attempt to develop other retinoid chemotypes for potential therapeutic applications, a series of analogues were designed that modified the ring with (bi)cyclohexenyl moieties derived from natural or modified monoterpenes while keeping the polyene side-chain of 9-*cis* geometry intact (Scheme 39).

Scheme 40. Synthesis of Retinoids by C8–C9 Bond Formation Using Cross-Coupling Reactions

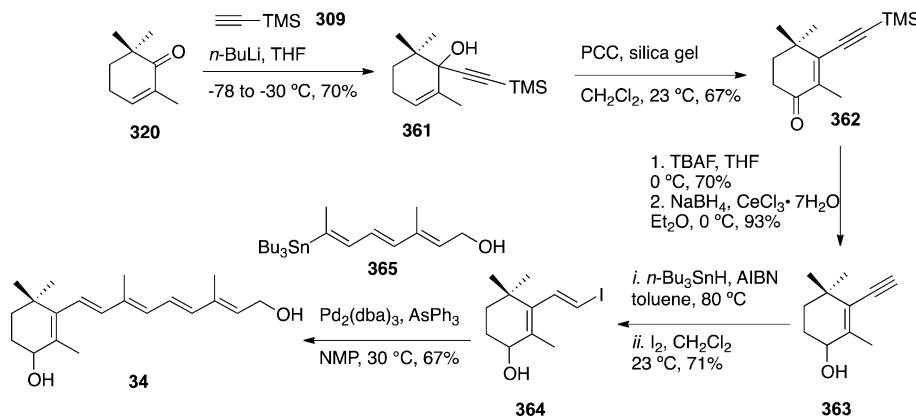
Scheme 41. Stereocontrolled Synthesis of (*S*)-9-*cis*-13,14-Dihydro-4-oxoretinoic Acid by C8–C9 Bond Formation Using the Stille Cross-Coupling Reactions

Although they share a common biogenetic precursor, the cyclic retinoid moiety is not made from cyclization of geranyl diphosphate in the presence of cyclases, as in the case of the monoterpenes. These “terpene–retinoid” chimeras with the hydrophobic ring derived from monoterpenes such as (−)-menthone, (+)-pulegone, (+)-camphor, (−)-*cis*-verbanone, (+)- and (−)-carvone, were stereoselectively synthesized using a Suzuki cross-coupling of boronates 340 and tetraenyl iodide 336 (Scheme 38), both generated *in situ*, as the key C6–C7 bond construction step.⁴⁰³ Similarly, application of the Stille reaction to the triflates 342 derived from the same and related terpenes, using CsF and/or CuI as additives, resulted in a series of ethyl 9-*cis*-retinoate analogues 343 and 341 with trienyl or tetraenyl side-chains, respectively (Scheme 39).⁴⁰⁴

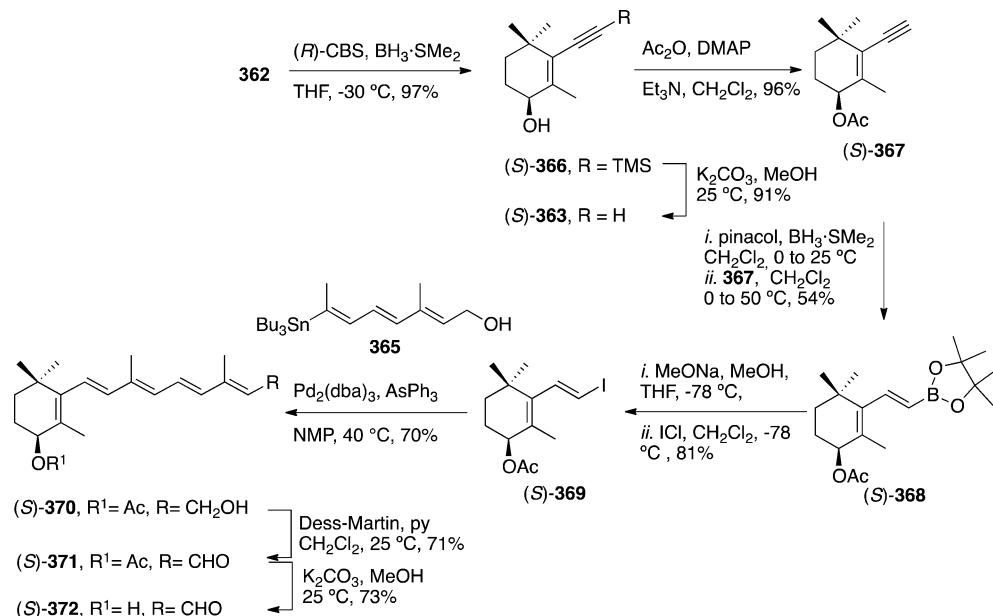
8.3.3. Formation of the C8–C9 Bond. In this variant, the Stille reaction of C₁₁ dienyl iodide 348 and C₉ trienylstannane 350 under Farina’s conditions led to ethyl all-*trans*-retinoate (237) at moderate temperatures (50 °C). Moreover, the preparation of dienyl iodide 348 from β-cyclocitral (14) faced difficulties due to the low reactivity of the aldehyde, and alternative procedures for the preparation of C₁₁ electrophiles from 351 were developed. Under modified conditions (2 equiv of CsF as an additive), dienyltriflate 352 (nonaflates have been used with similar success)⁴⁰⁵ underwent Stille coupling with *cis*-stannyltrieneester 344 (40–45 °C) to afford ethyl 9-*cis*-retinoate (254) in 88% yield (Scheme 40).⁴⁰⁶

The stereocontrolled synthesis of (*S*)-9-*cis*-13,14-dihydro-4-oxoretinoic acid (*S*)-39 and its enantiomer featured a Stille coupling of dienyl iodide 355 and dienylstannane 357 (Scheme

Scheme 42. Stereocontrolled Synthesis of all-*trans*-4-Hydroxyretinol (34) by C8–C9 Bond Formation Using the Stille Cross-Coupling Reactions



Scheme 43. Enantioselective Synthesis of (*S*)-all-*trans*-4-Hydroxyretinol by C8–C9 Bond Formation Using the Stille Cross-Coupling Reactions



41).⁴⁰⁷ For the synthesis of 355, alcohol 353 was transformed into the allylic chloride, which underwent Arbuzov reaction with neat trimethyl phosphite and an excess of sodium iodide to provide phosphonate 354 in 61% overall yield. HWE olefination between the semistabilized phosphonate 354 and enantiopure (*S*)-298 using NaHMDS provided the iodide (*S*)-355 with high stereoselectivity (*E/Z* 14:1, 38% yield). The use of modified Stille cross-coupling reaction conditions⁴⁰⁸ involving catalytic amounts of Pd(PPh₃)₄ and CuTC⁴⁰⁹ in DMF in the presence of (NBu₄)(Ph₂PO₂) as tin scavenger⁴¹⁰ afforded, after 30 min at 25 °C, tetraenol 358 in excellent yield (91%) from the coupling of 357 and (*S*)-355. Oxidation of the mixture of diastereomers 358 to the corresponding ketone with MnO₂ under basic conditions and hydrolysis of (*S*)-359 with aqueous KOH in MeOH produced (*S*)-39 in 73% yield.⁴⁰⁷

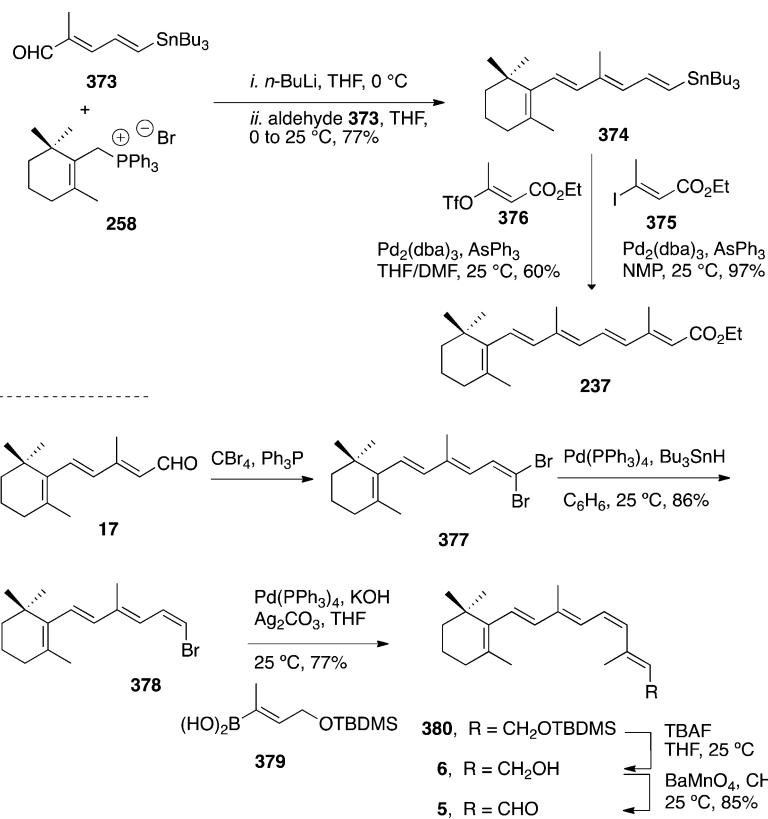
An alternative route to the enantiomers of (*S*)-9-*cis*-13,14-dihydro-4-oxoretinoic acid⁴⁰⁷ was explored on the basis of the Julia–Kocienski condensation of a Z-iodo-benzothiazolyl- (BT) or phenyltetrazolyl (PT)-sulfone 360 with the same aldehyde (*S*)-298³⁶⁸ but a mixture of the four possible double-bond

isomers with the 9-*cis*,11-*cis* isomer of dienyliodide (*S*)-355 as major product was obtained (Scheme 41).⁴⁰⁷

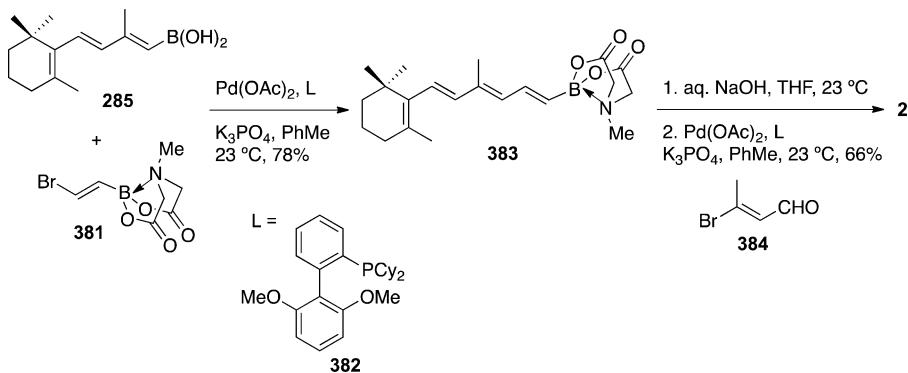
Enynone 362 was used as the substrate for the synthesis of C4-hydroxyretinoids as racemates and in enantiopure form. For the preparation of ketone 362, the lithium anion of TMS-acetylene (*n*-BuLi, THF) was added to cyclohexenone 320. The allylic transposition of the intermediate chromate ester obtained upon treatment of propargylic alcohol 361 with PCC in silicagel, and subsequent oxidation of the secondary alcohol, provided 362 in 67% yield (Scheme 42). Deprotection of enynone 362 with TBAF and treatment with Luche' reagent (NaBH₄/CeCl₃) furnished 363. The reaction of 363 with Bu₃SnH and AIBN in toluene at 80 °C, and in situ exchange with iodine, afforded the stereochemically homogeneous dienyliodide 364 with *trans* geometry. Under Farina's conditions, the coupling of 365 and 364 furnished 4-hydroxyretinol (34) in 67% yield.⁴⁰⁰

The synthesis of the enantiomers of 4-hydroxyretinal was carried out using as a key step the Stille cross-coupling of the same trienylstannane 365 and enantiopure dienyliodides 369.³⁶⁰ The configuration of this fragment (only the *S*

Scheme 44. Stereocontrolled Synthesis of Retinoids by C12–C13 Bond Formation Using Pd-Catalyzed Cross-Coupling Reactions



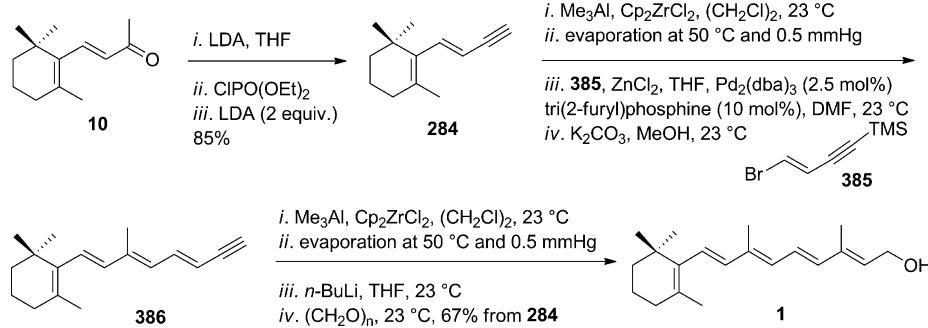
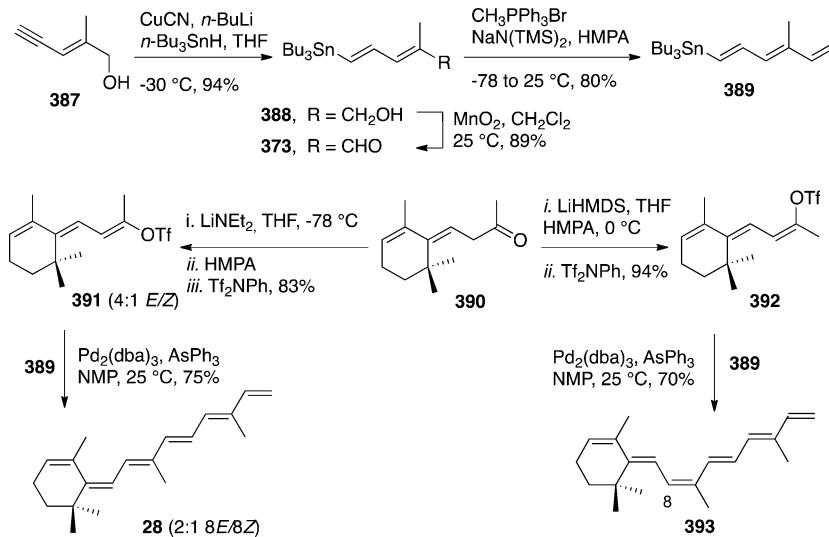
Scheme 45. Iterative Pd-Catalyzed Cross-Coupling Reactions (ICC) for the Synthesis of All-trans-retinal (2)



enantiomer is shown in Scheme 43) derives from the CBS reduction of enynone 362. Treatment of 362⁴⁰⁰ with (R)-2-methyl-CBS-oxazaborolidine and BH₃·SMe₂ in THF at -30 °C afforded alcohol (S)-366 in excellent yield (97%). The regio- and stereoselective hydroboration of the derived acetate (S)-367 using pinacol borane provided pinacolboronate (S)-368 (54% yield). Boron–halogen exchange with retention of configuration was achieved by treatment of (S)-368 with MeONa at -78 °C followed by addition of ICl. Coupling of iodide (S)-369 with stannanyltriene 365 under Farina's conditions afforded (S)-370 in 70% yield. Oxidation of alcohol (S)-370 was carried out with the Dess–Martin periodinane in CH₂Cl₂–pyridine to minimize isomerization at the terminal double bond. Despite these precautions, an 8.5:1 mixture of the all-trans and 13-cis isomers of (S)-371 was obtained in 71% yield. Additional isomerization of the C₁₃–C₁₄ bond took place

upon deprotection of the acetates (S)-371 with K₂CO₃ in MeOH, and led to (S)-372 as a 3:1 mixture of trans/13-cis isomers (Scheme 43).³⁶⁰

8.3.4. Formation of the C12–C13 Bond. The C12–C13 bond construction uses functionalized fragments with tetraenyl and vinyl structures. The C₁₆-tetraenyl stannane 374 was prepared in 77% yield by Wittig condensation of phosphonium salt 258 and stannyl aldehyde 373. Coupling of 374 with C₄-alkenyl iodide 375 took place at room temperature under Farina's conditions to afford ethyl all-trans-retinoate (237) in 97% yield. Triflates are convenient synthetic equivalents, and the use of 376 prepared from ethyl acetoacetate provided 237. This sequence can be extended to the synthesis of C13-substituted analogues using triflates prepared from other β-ketoesters (Scheme 44).⁴¹¹

Scheme 46. Stereocontrolled Synthesis of All-trans-retinol (**1**) by Consecutive C10–C11 and C14–C15 Bond Formation**Scheme 47.** Stereocontrolled Synthesis of Anhydroretinol and Its 8Z Isomer by Stille Cross-Coupling Reactions

The halogen-selective Pd-catalyzed hydrogenolysis of 1,1-dibromotetraene **377** (obtained from **17** using the Corey–Fuchs protocol) with Bu₃SnH in the presence of (PPh₃)₄Pd in benzene provided stereodefined (Z)-1-bromotetraene **378**.⁴¹² Suzuki cross-coupling of **378** with alkenylboronic acid **379** using the rate-enhancement effect of silver salts afforded protected 11-cis-retinol **380**, which can be converted uneventfully into **6** and **5** (Scheme 44).

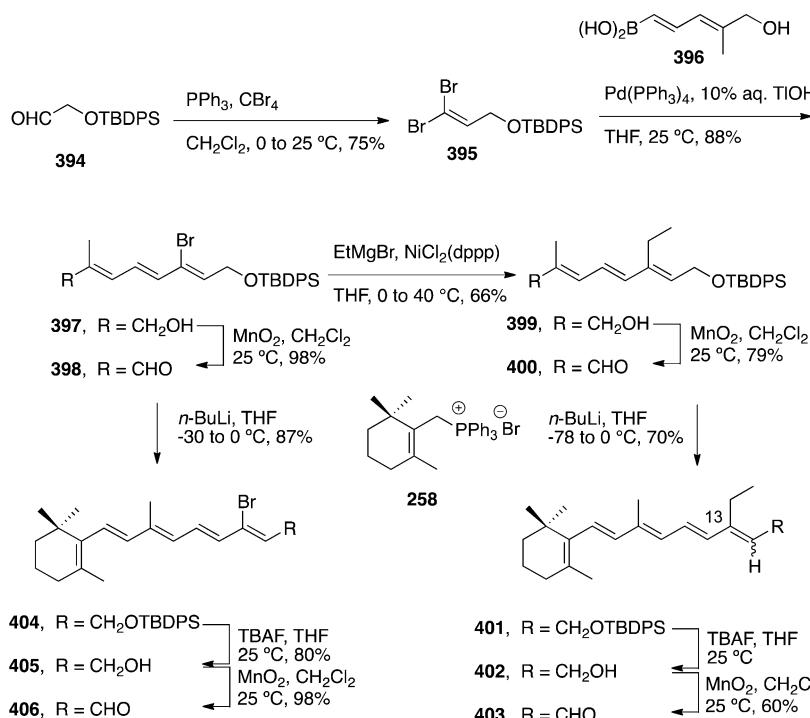
A modular synthesis of all-trans-retinal (**2**) utilizes the iterative cross-coupling (ICC) of *B*-protected haloalkenylboronates such as MIDA boronate **381**, which derives from the treatment of the boronic acid with *N*-methyliminodiacetic acid (MIDA).⁴¹³ The MIDA boronates are stable to most reaction conditions and are transformed back into the boronic acids by treatment with base. For the application of the ICC protocol, after Suzuki coupling of **285** with the halide end of bisfunctionalized building block **381** to give **383**, the boronic acid was unmasked by base treatment, and then coupled with C4 fragment **384** to produce **2** (Scheme 45). The reaction conditions of the first Suzuki coupling avoid the use of strong inorganic bases to preserve the MIDA boronate intact, and the reaction is best performed using bulky ligands at Pd (**382**).⁴¹³

8.3.5. Formation of the C14–C15 Bond. In addition to its efficient coupling with C₆ electrophiles of *E* and *Z* configurations, the C₁₄ component obtained by methylalumination/transmetalation of **284** (compound **285**, Scheme 32) can also be used in Negishi cross-coupling with C₄ bromoenyne **385** to produce after deprotection the C₁₈ tetraenylstannane **386**. A

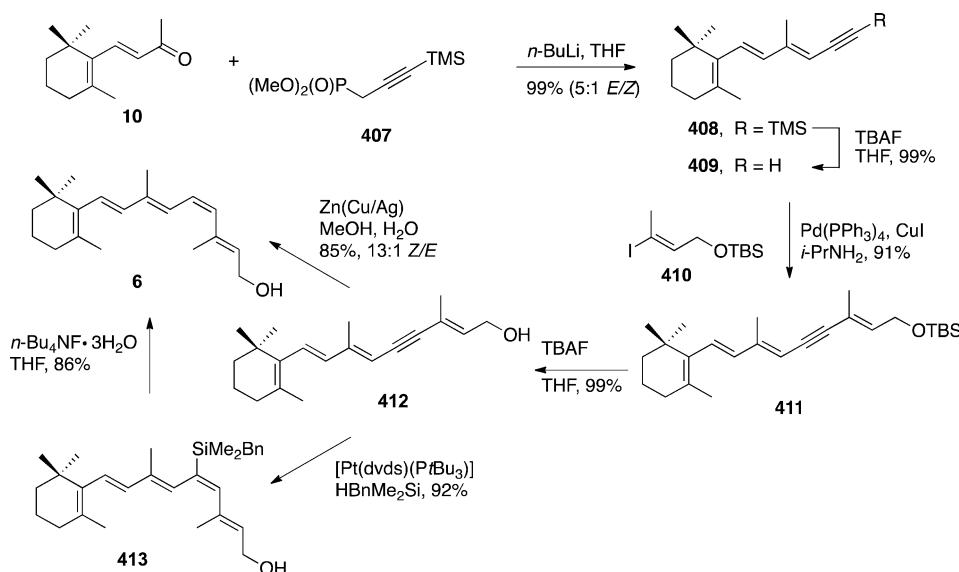
longer C₁₉ organometal component can be analogously obtained from **386**, and this intermediate can be treated with a C1 component to prepare retinoids. This tactic has been applied to the stereoselective preparation of all-trans-retinol (**1**) in three linear steps and 40% overall isolated yield (Scheme 46).⁴¹⁴ Alkyne methylalumination of **284**,³⁷⁶ in situ transformation into the alkenylaluminate complex, exchange to zinc, and Pd-catalyzed cross-coupling of the organozinc with C₄ bromoenyne electrophile **385** provided tetraenylstannane **386**. Another methylalumination of **386** and trapping the alkenylaluminate complex with formaldehyde completed the retinoid side-chain of vitamin A (**1**).

Retinoids with more extended unsaturated chains have also been prepared using Pd-catalyzed cross-coupling processes. The hexaene anhydroretinol (**28**) and its (8*Z*)-isomer (**393**) were synthesized using the Stille cross-coupling of C₁₃ and C₇ triene fragments (Scheme 47).⁴¹⁵ Formally, the construction of the *retro*-retinoid structure of anhydroretinol requires the use of unnatural disconnection patterns, in this case a C9–C10 single bond formation. The preparation of the C₇ trienylstannane **389** involved a regio- and stereoselectively stannylcupration/protonolysis of enynol **387**, oxidation of **388** to the corresponding aldehyde **373**, and Wittig olefination at low temperature, all of the steps proceeding in good yields. The preparation of stereodefined triflates **391** and **392** proved to be more challenging, and extensive experimentation using different ionone isomers, bases, and trapping agents was required to find optimal conditions. The best results were obtained starting

Scheme 48. Stereocontrolled Synthesis of C13-Substituted Retinals by Suzuki and Kumada–Tamao–Corriu Cross-Coupling Reactions



Scheme 49. Stereocontrolled Synthesis of 11-cis-Retinoids from 11,12-Didehydroretinoids by Hydrogenation and Pt-Assisted Hydrosilylation/Desilylation



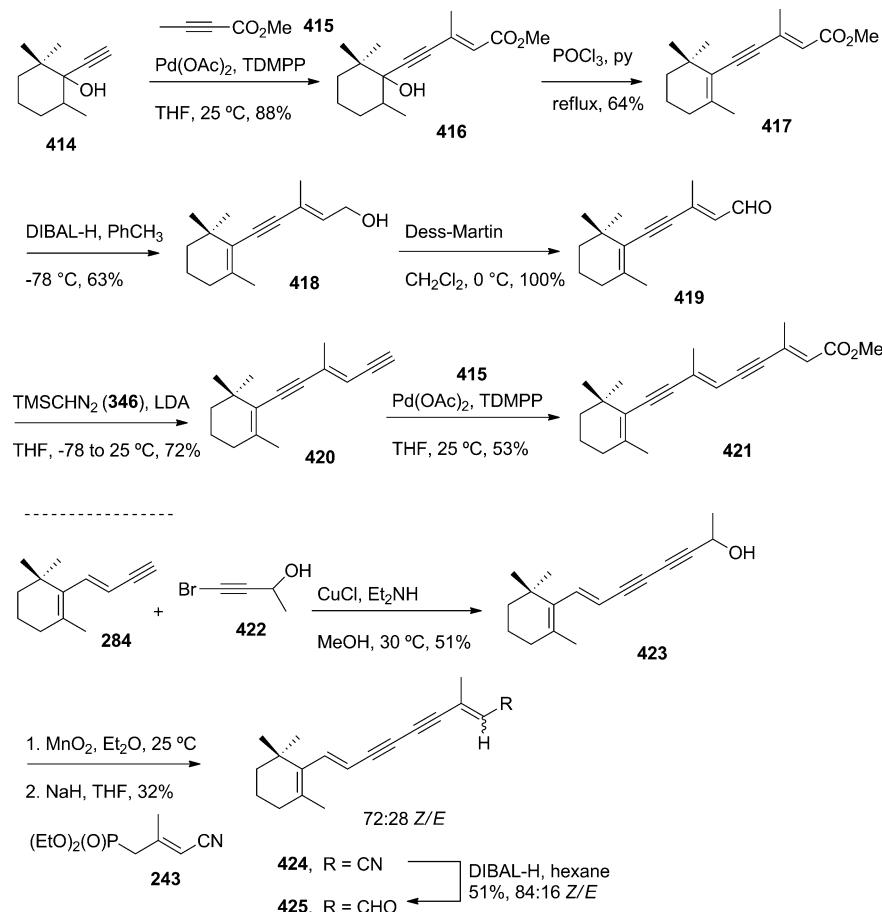
from *retro-ionone* (**390**). (*Z,E*)-Triflate **392** was stereo-selectively produced in 94% yield by deprotonation of **390** with LHMDS in THF at 0 °C in the presence of HMPA, followed by trapping the enolate with *N*-phenyltriflimide. The best conditions found for the preparation of (*E,E*)-triflate **391** (as a 4:1 mixture of isomers in 83% yield) were the sequential treatment with LiNEt₂, addition of HMPA, and trapping the lithium enolate with *N*-phenyltriflimide. The choice of Farina's conditions for the cross-coupling of the fragments afforded (8*Z*)-anhydroretinol **393** in 70% yield, and anhydroretinol (**28**) together with its (8*Z*)-isomer (**393**) (ca. 2:1 ratio) in 75% yield, respectively (Scheme 47). The partial isomerization was shown

to take place at the product stage due to the instability of these highly conjugated polyenes.^{381,397}

On the other hand, the dehydration of vitamin A (**1**) induced by acid (HCl, EtOH, 25 °C, 30 min) is a more traditional method for preparation of anhydroretinol. Although convenient for its simplicity, the low yield and lack of stereocontrol are drawbacks of the procedure, because mixtures of anhydroretinol isomers are routinely obtained in 10–30% yields.⁷⁷

8.3.6. Other Metal-Catalyzed Processes. A Kumada–Tamao–Corriu⁴¹⁶ cross-coupling reaction was used for the incorporation of the ethyl group at position C13 in the stereoselective synthesis of all-*trans*-13-demethyl-13-ethylretinal

Scheme 50. Synthesis of Didehydro and Tetrahydroretinoids



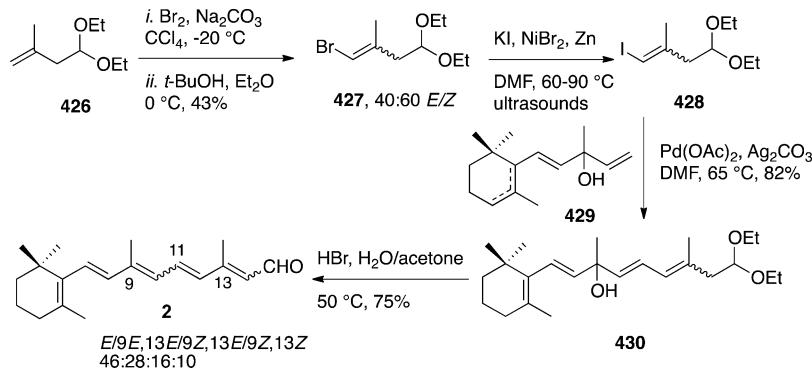
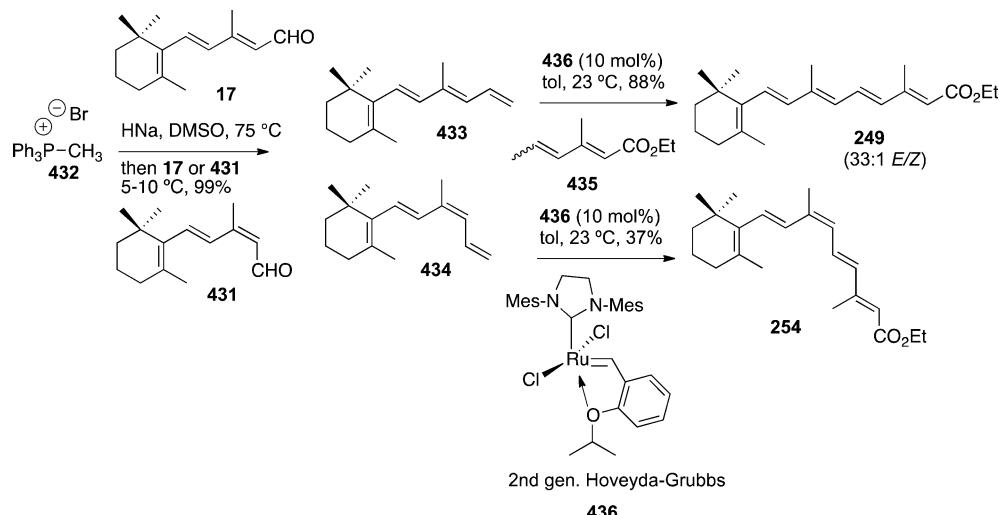
403 (Scheme 48).^{411a,417} The synthesis moreover uses the preferential reactivity of (*E*)-halides in alkenyl *gem*-dibromides in Suzuki reactions.⁴¹⁸ Because of the rate differences between the *Z* and *E* electrophiles in favor of the latter, dibromide **395**, obtained from aldehyde **394** in 75% yield, reacted with boronic acid **396**⁴¹⁹ under mild conditions to afford the bromotrienol **397** in 88% yield. Cross-coupling of bromide **397** and ethylmagnesium bromide catalyzed by NiCl₂(dppp) and subsequent oxidation of the alcohol obtained afforded trienal **400** in good yield. Wittig olefination of **400** with phosphonium salt **258** produced with complete selectivity alcohol **401** in 70% yield, which was deprotected with TBAF and the alcohol **402** oxidized with MnO₂ under basic conditions to furnish all-*trans*-13-demethyl-13-ethylretinal (**403**). This analogue is configurationally less stable than the parent system, because a 2:1 ratio of the 13*E*/13*Z* isomers was obtained.⁴¹⁷ The same intermediate **397** was used for the synthesis of (13*Z*)-13-bromo-13-demethylretinal (**406**). Oxidation of **397** to trienal **398** followed by its reaction with the anion of phosphonium salt **258** afforded **404** in 87% yield (Scheme 48). Deprotection of the alcohol followed by oxidation of **405** with MnO₂ provided another unnatural retinoid, (13*Z*)-13-bromo-13-demethylretinal (**406**), which have been used in studies of bR artificial pigment properties.^{411a}

Dihydroderivatives that include a triple bond replacing some of the retinoid chain double bonds have been prepared primarily by application of the Sonogashira cross-coupling reaction.⁴²⁰ 11,12-Didehydroretinol (**412**) is a particularly valuable analogue because it can be used as a precursor of

11-*cis*-retinoids (Scheme 49).⁴²¹ The semihydrogenation of the triple bond of **412** to 11-*cis*-retinol (**6**) using Lindlar's catalyst⁴²² is often capricious, but could be achieved with Cu/Ag-activated Zn dust in MeOH/H₂O as reagent (13:1 *Z/E*, 85%).³⁸⁷ Terminal trienye **409** was obtained by a sequence that starts with the HWE condensation of β-ionone (**10**) and the anion of dimethyl (3-trimethylsilyl-2-propynyl)-phosphonate (**407**). Compound **408** (99% yield) obtained with moderate *E/Z* stereoselectivity (5:1) was deprotected with TBAF (99% yield) and the product coupled with vinyl iodide **410** in the presence of Pd(PPh₃)₄, CuI, and *i*-PrNH₂ to afford 11-yne precursor **411** in 91% yield. Another deprotection with TBAF in quantitative yield afforded the hydrogenation substrate **412**.

An alternative to the stereoselective preparation of 11-*cis*-retinoids from the 11,12-didehydroretinal analogues⁴²³ is based on the regio- and stereoselective Pt-mediated hydrosilylation/protodesilylation of **412** with Karstedt's catalyst [Pt(dvds)(Pt-Bu₃)] (dvds = divinyltetramethyldisiloxane) to give **413** in high yield (Scheme 49) followed by fluoride-induced cleavage. Moreover, this chemoselective net *syn* reduction can be executed in just one step from the corresponding internal 11-yne substrate **412** and is amenable to isotopic labeling in one or both positions of the 11-*cis*-retinoid product, using the corresponding deuteriosilane and/or D₂O as reagents.⁴²³

An atom-economical alternative to enynes is the Pd-catalyzed cross-coupling reaction of alkynes with activated internal alkynes. This mild method for C–C bond formation, which is promoted from the C–H insertion intermediate, has been

Scheme 51. The Heck Reaction of Vinyl Fragments in the Construction of the Retinoid Side-Chain**Scheme 52.** The Acyclic Cross-Metathesis Reaction in the Construction of the Retinoid Side-Chain

applied to the preparation of conformationally rigidified analogues of all-*trans*-retinoic acid such as methyl 7,8,11,12-tetradehydroretinoate (**421**) (Scheme 50).⁴²⁴ The coupling of alkyne **414** (a mixture of diastereomers) and methyl 2-butynoate **415** catalyzed by Pd(OAc)₂ and the bulky tris(2,6-dimethoxyphenyl)phosphine (TDMPP) in THF at ambient temperature afforded ynoate **416** in 88% yield. Dehydration of **416** to **417** by treatment with POCl₃ and elimination (64%), followed by reduction of **417** with DIBAL-H to alcohol **418** (63%), oxidation to aldehyde **419** in 87% yield using the Dess–Martin reagent, and alkyne chain extension with trimethylsilyldiazomethane (**346**) provided terminal alkyne **420** (72%). A second C–H insertion–addition sequence applied to alkyne **420** and **415** furnished diyne **421** in 53% yield.

Alkynes have also replaced trisubstituted olefins in retinoids, as in 9,10-didehydro-19-norretinal and 9,10,11,12-tetradehydro-19-norretinal **425**. The latter was prepared by Cadot–Chodkiewicz reaction⁴²⁶ of dienyne **284** and bromoalkyne **422** catalyzed by CuCl in the presence of Et₂NH (51% yield).⁴²⁵ Oxidation of **423** with MnO₂ and HWE condensation of the derived aldehyde with **243** produced in 32% combined yield nitrile **424**, which was reduced with DIBAL-H to give **425** as an 84:16 mixture of the terminal double-bond isomers (Scheme 50). Other analogues with diynes replacing alternative dienes of the retinoid side-chain have also been prepared.⁴²⁷

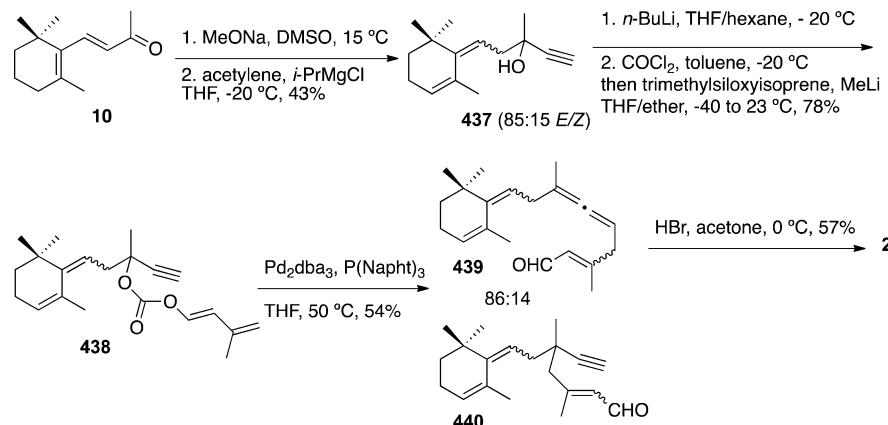
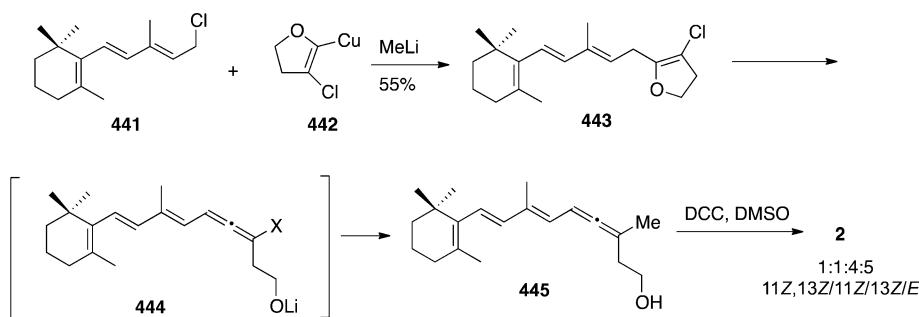
A synthesis of all-*trans*-retinal (**2**) used a Heck reaction for the construction of the C₂₀ skeleton.⁴²⁸ In contrast to the Pd-catalyzed cross-coupling reactions, the Heck alkenylation⁴²⁹

faces limitations for its application to the synthesis of polyenes due to competing stereoisomerization and other side reactions, such as β -elimination, as well as lack of control of the regioselectivity. However, dienes can be formed by Heck vinylation reactions of tertiary allylic alcohols. If vinyl iodides derived from C₅-acetals are used as the other component in Heck coupling, the resulting ω -hydroxy acetal can subsequently be deprotected by acid treatment and dehydrated to provide conjugated polyenals.⁴²⁸ In the application of the method to retinal, the vinyl iodide **428**, obtained from the bromide **427** as a mixture of *E/Z* isomers in a 40:60 ratio, was treated with the C₁₅ allylic alcohol **429** (a mixture of positional isomers) in the presence of a catalytic amount of palladium acetate and stoichiometric amounts of silver or thallium salts, to give the condensation product **430** in good to excellent yields (82% with Ag₂CO₃ at 65 °C for 3 h) as mixtures of isomers reflecting the composition of the starting iodide (Scheme 51). Treatment of **430** with dilute hydrobromic acid in aqueous acetone at 50 °C gave mixtures of diastereoisomers of **2** where the all-*trans* isomer predominated (46:28:16:10).

8.4. Formation of the Polyene Chain by Cross-Metathesis Reactions

The olefin metathesis reaction³⁰¹ is one of the most general and widely applicable synthetic method for Csp²=Csp² bond formation, as shown by its presence as a key step in the synthesis of a great variety of natural products.⁴³⁰ However, the extension of this methodology to conjugated polyenes⁴³¹ is not

Scheme 53. Isomerization of Bis-retro-retinoids to Retinoids

Scheme 54. Isomerization of *retro*-Retinal

fully developed due to concerns about the control of the site-selectivity and stereoselectivity of the metathesis and the stability of the polyenes to the reaction conditions. In early reports, the cross-metathesis of β,β -carotene 4 with ethylene or methyl sorbate led to complex mixtures of products in low yields.⁴³² Improvements on catalyst design and selection of partners made it possible to extend the methodology to conjugated polyenes such as carotenoids⁴³³ and also to retinoids.⁴³⁴ Thus, ethyl all-*trans*-retinoate (249) and its 9-*cis* isomer 254 were efficiently prepared by the acyclic cross-metathesis of tetraenes 433 and 434, respectively (obtained by Wittig methylenation of the precursor aldehydes 17 and 431 using dimsyl sodium to generate the ylide from 432), with ethyl 3-methylhexa-2,4-dienonate 435 promoted by the second-generation Hoveyda–Grubbs catalyst 436 (Scheme 52). Because the olefin metathesis initiators are sensitive to steric hindrance, the cross-metathesis processes at the terminal double bond are favored over those of the tetra-, tri-, and disubstituted double bonds in the starting tetraenes 433 and 434.^{430c}

8.5. Formation of the Polyene Chain by Rearrangement Reactions

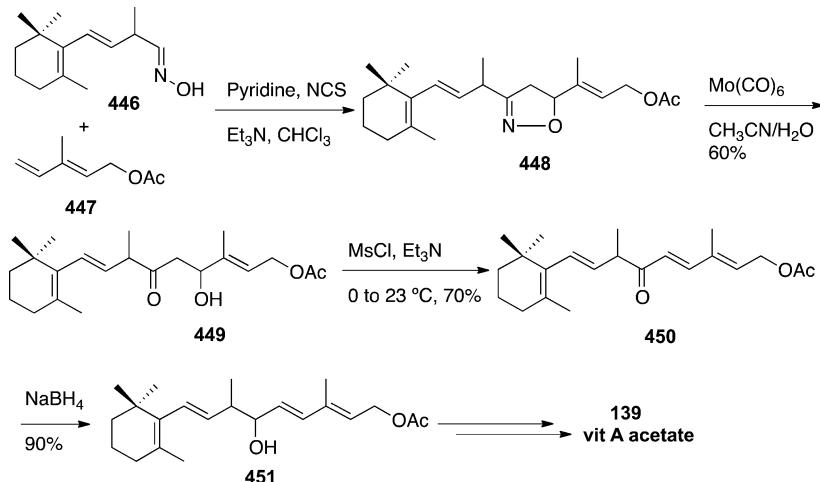
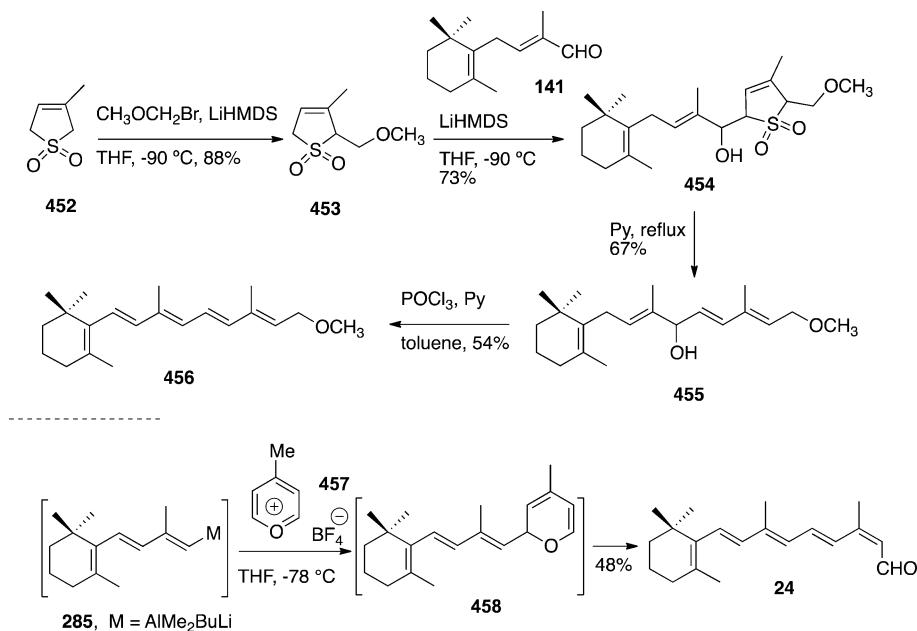
8.5.1. Decumulation of Allene(*retro*)-retinoids. Allenes with *retro*-retinoid or double *retro*-retinoid structures can be isomerized to the fully conjugated polyenes. For example, allenal 439 with a double *retro*-retinoid structure underwent isomerization to the conjugated pentaenal of all-*trans*-retinal (2).⁴³⁵ The sequence started with the deconjugation of β - to *retro*- α -ionone and addition of the Grignard reagent prepared from acetylene to the latter to provide propargylic alcohol 437 (Scheme 53). Treatment of alcohol 437 with phosgene and trimethylsiloxyisoprene provided the mixed carbonate 438.⁴³⁵

The palladium-induced rearrangement of 438 using Pd(PPh₃)₄ and tris-naphthyl phosphine generated a mixture of the rearranged allenyl/propargyl products (86:14 439/440, 54% yield) after the intramolecular capture of an intermediate allenyl/propargylpalladium with the pendant enoether. The allenal 439 was isomerized in acidic media to afford retinal as a 75:25 mixture of geometric isomers at both trisubstituted double bonds.⁴³⁵ This mixture was isomerized to the *trans* isomer 2 by treatment with iodine, acids, or heat under equilibrating conditions using the ease of crystallization of the all-*trans*-retinal/hydroquinone complex.⁴³⁵

The conjugated polyene skeleton of retinoids can also be constructed by isomerization of β,γ -allenic aldehydes.⁴³⁶ Thus, Moffatt oxidation of 14,12-*retro*-retinol 445 with DCC in the presence of DCC afforded in 55% yield a mixture of the four stereoisomeric retinals at the C11=C12 and C13=C14 bonds in a 1:1:4:5 ratio (Scheme 54). The 12,14-*retro*-retinol structure of 445 was obtained from the MeLi-induced rearrangement of 5-substituted 4-chloro-2,3-dihydrofuran 443, the substitution product of chloride 441 by the 3-chloro-4,5-dihydro-2-furylcopper species 442.⁴³⁶

8.5.2. Thermal Pericyclic Reactions. Pericyclic reactions have been used for the construction of the polyene chain of retinoids (using 3 + 2 cycloaddition), for the temporary protection of a latent diene (retrochelotropic reactions of sulfolenes), and for the positional exchange of hydrogen atoms within cumulated analogues by thermally induced [1,j]-H sigmatropic rearrangements. Fragments (dienes, trienes) of the final polyene skeleton have also been made by sigmatropic rearrangements.⁴³⁷ Because of the polyenic nature of the retinoids, some of the *cis* isomers can undergo pericyclic–pericyclic sequential transformations, including [1,j]-H sigma-

Scheme 55. Cycloaddition of Nitrile Oxides and Alkenes for the Retinoid Side-Chain Construction

Scheme 56. Connective Sulfolene Alkylation/Retrochelotropic Ring-Opening and Electrocyclic Ring-Opening of (2*H*)-Pyrans for the Retinoid Side-Chain Construction

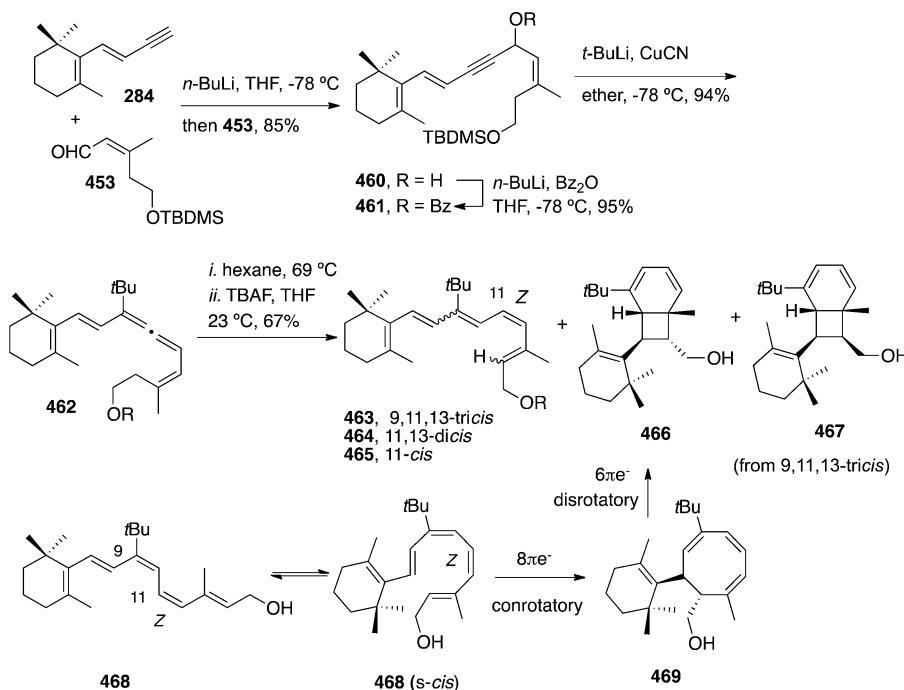
tropic shifts and electrocyclic reactions. Pericyclic processes of retinal derivatives have been proposed to take place in nature. A [1,5]-H sigmatropic shift and an electrocyclic reaction of a formal dieniminium ion could be involved in the formation of the fluorescent amphiphilic pyridinium bis-retinoid pigment A2-E (**58**) and of dihydropyridine analogues (**62, 63**) from two molecules of retinal (see section 5) at physiological temperature in the retina.

The (3 + 2) dipolar cycloaddition is a highly convergent approach to the side-chain of retinoids and arotenoids, as it was found that the nitrile oxide intermediate resulting from the treatment of oxime **446** with NCS in pyridine added to diene **447** to provide regioselectively isoxazoline **448** in good yield (Scheme 55). Reduction of the isoxazoline ring of **448** was accomplished by treatment with Mo(CO)₆, and the β-hydroxyketone **449** was dehydrated to furnish unsaturated ketone **450**. The derived alcohol **451** was transformed uneventfully into vitamin A acetate (**139**).⁴³⁸

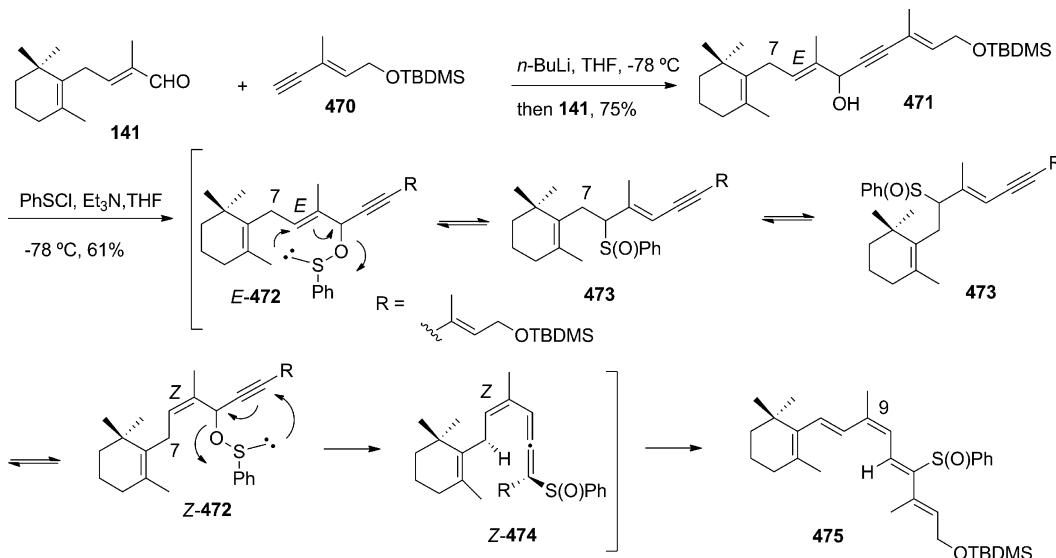
The cheletropic extrusion of sulfur dioxide from five membered ring sulfolenes unmasks a stereodefined diene if the process occurs with rotational selectivity. 2-Methylsulfolene (**452**) can be considered a synthetic equivalent of an isoprene unit, and is substituted regioselectively at the most hindered position by alkylation of the anion at -90 °C with bromomethyl methyl ether (Scheme 56). Addition to the C₁₄ aldehyde **141** of the anion of the derived C₆ sulfolene **453** at the less hindered site provided the C₂₀ retinoid skeleton of **454**. The sulfur dioxide extrusion of **454** led to tetraene **455**, which was treated with POCl₃ and pyridine in CHCl₃ to afford methyl all-*trans*-retinyl ether (**456**) in 54% yield as a single isomer.⁴³⁹

A unidirectional electrocyclic ring-opening of a (2*H*)-pyran **458** resulting from the position-selective addition of an organometallic reagent to an 4-alkylpyrylium salt **457** afforded alkyl-substituted dienals with (2*Z*,4*E*) geometry. For application of the methodology to retinoid synthesis, the ate complex **285**, obtained by the treatment of precursor alane with *n*-BuLi at -78 °C, was added to 4-methylpyrylium tetrafluoroborate

Scheme 57. The Approach to 11-*cis*-Retinoids Using [1,5]-H Sigmatropic Rearrangement of Vinylallenenes (10,14-*retro*-Retinoids)



Scheme 58. A Stereoselective Pericyclic Cascade to 9-*cis*-Retinoids Using Sigmatropic Rearrangements with the Involvement of Allyl/Propargyl Sulfenates and Allyl/Allenyl Sulfoxides

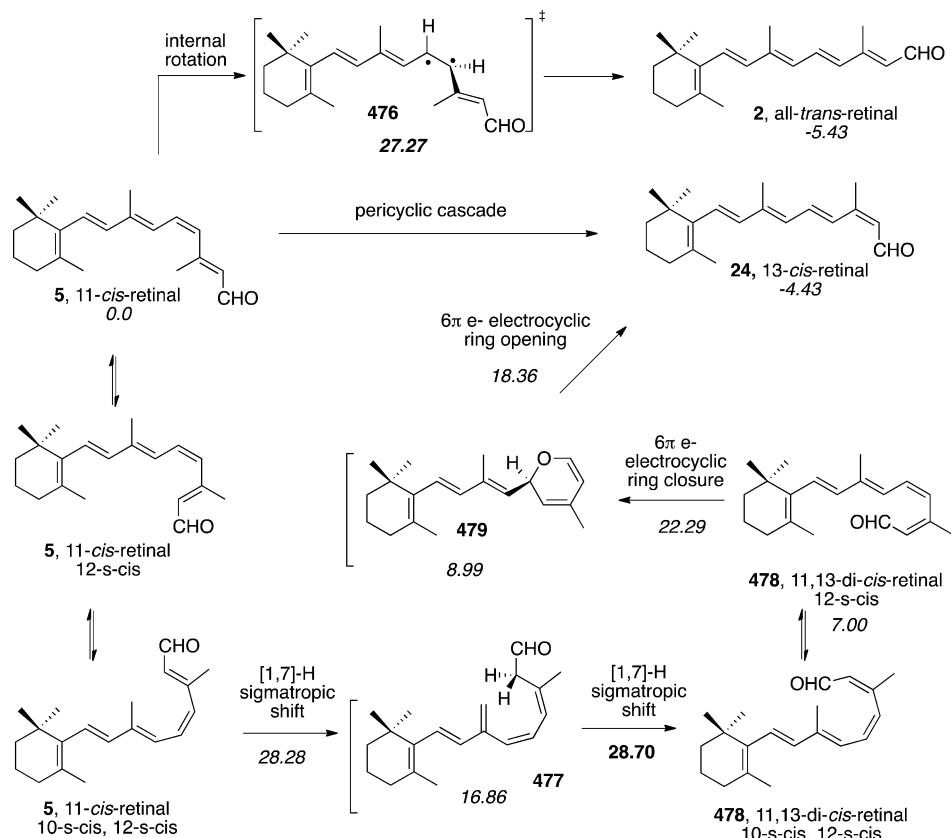


457, which functions as an equivalent of a C₆ homologation building block, to afford (13Z)-retinal (**24**) in 48% yield (Scheme 56).⁴⁴⁰

Although the temperatures required to induce [1,*j*]-H sigmatropic rearrangements in conjugated systems are generally high, in the case of allenes such rearrangements take place below 100 °C. Moreover, these are irreversible processes due to the lower steric hindrance to hydrogen migration to an allene center when compared to a Csp² atom, and to the energy released upon “decumulation” of the system. Sigmatropic rearrangements of vinylallenenes⁴⁴¹ are classical stereoselective approaches to the triene and pentaene fragments of vitamin D and 11-*cis*-retinol, and have also proven useful to obtain other

cis isomers of retinoids and 11-*cis*-retinol analogues. For example, the thermal rearrangement of the dienyl-vinylalene **462** at 69 °C for 22h and deprotection with TBAF afforded a mixture of products (Scheme 57) in 67% overall yield. In addition to the 9-*tert*-butyl-11,13-di-*cis*-retinol (**464**, 19%), its 9,11,13-tri-*cis*- (**463**, 7%) and 11-*cis*-retinol isomers (**465**, 10%), bicyclo[4.2.0]octa-2,4-dienes **466** and **467** (11 and 20% yield) were also isolated and characterized. These originate from additional rearrangements of the 9,11-di-*cis*- (non isolated) and 9,11,13-tri-*cis* isomers. Two torquoselective electrocyclic ring closure reactions, first a 8π⁻ conrotatory cyclization from the *s-cis* conformer of **468** to give cyclooctatriene **469**, and second a 6π⁻ disrotatory cyclization of the latter, could mechanisti-

Scheme 59. A Direct Bond Rotation to All-*trans*-retinal and a Pericyclic Cascade to 13-*cis*-Retinal Are Compatible with DFT Studies To Explain the Thermal Rearrangement of 11-*cis*-Retinal, the Chromophore of the Visual Pigment^a



^aEnergies (B3LYP, in kcal/mol) are given for the minima and the transition structures.

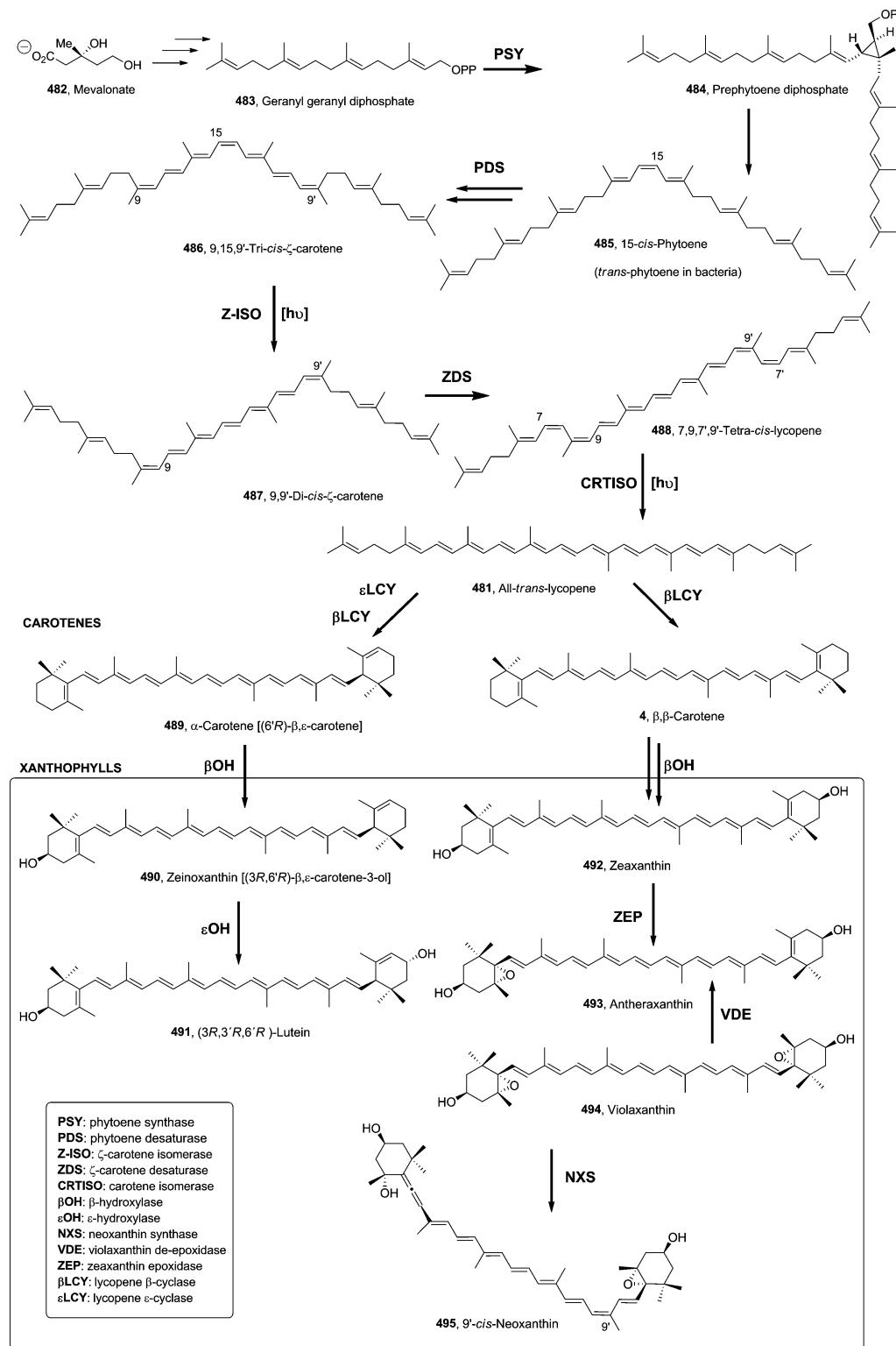
cally explain the generation of bicyclo[4.2.0]octa-2,4-diene **466** (Scheme 57). Allene **462** was made by the regioselective (S_N2') displacement of a propargylic benzoate **461** (obtained from the propargylic alcohol **460**, itself made by addition of the alkynyl anion of **284** to C_6 aldehyde **453**) by a bulky *tert*-butyl cyanocuprate reagent.⁴⁴²

A pericyclic cascade was proposed to explain the stereoselective formation to 9-*cis*-retinoids **475** from the reaction of alkenynol **471** with arylsulfenyl chloride (Scheme 58).⁴⁴³ The process comprises an ordered sequence of sigmatropic rearrangements: a reversible [2,3]-allyl sulfenate to allyl sulfoxide shift, which interconverts the isomers *E*-**472** and *Z*-**472**, followed by a [2,3]-rearrangement of the propargyl sulfenate with *Z* geometry to allenyl sulfoxide *Z*-**474**, and a final stereodifferentiating [1,5]-H sigmatropic migration leading to polyene **475**.⁴⁴⁴ The migration of hydrogen from C7 to C11 was demonstrated by labeling experiments.

The double diastereoselection of the [1,5]-H sigmatropic shift to afford a single isomer of the final polyene **475** is thought to arise from a combination of the electronic effect of the sulfoxide at one terminus⁴⁴⁵ and the steric effect imparted by the bulky trimethylcyclohexenyl substituent at the other terminus. Thus, the cascade of sigmatropic rearrangements generates an *E,Z,Z*-triene fragment of the conjugated polyenic side-chain from alkenynol **471**. The complete control of the side-chain geometry of 9-*cis*-retinoids has been synthetically exploited, and C7,C11-doubly labeled and ring-modified 9-*cis*-retinoids have been prepared according to the general scheme indicated above for the parent compound 9-*cis*-retinol.⁴⁴⁴ The

role of the sulfoxide is to provide stereochemical control of the [1,5]-H rearrangement by directing the migrating H *anti* to its location.⁴⁴⁵ This group was then reduced stereoselectively with *t*-BuLi in the presence of MeOH in 40% yield.

Another pericyclic cascade has been proposed (Scheme 59), based on DFT studies, to account for the thermal isomerization of 11-*cis*-retinal to 13-*cis*-retinal.⁴⁴⁶ It was experimentally found that, upon heating to 80 °C, 11-*cis*-retinal (**5**) yields a mixture of all-*trans*-retinal (**2**) and its 13-*cis* isomer (**24**), which are not interconverted in the time course of the experiment. Density functional theory methods (B3LYP in conjunction with a 6-31+G(d,p) basis set, and expanding the SCF calculation to an unrestricted space, UB3LYP for the structures exhibiting unstable restricted wave functions) were used for the theoretical treatment of the system. The computed activation barriers for the 11-*cis*-retinal to all-*trans*-retinal isomerization ($\Delta G^\ddagger = 27.3$ kcal/mol at the B3LYP level) are close to the experimental value ($\Delta G^\ddagger = 26.2$ kcal/mol). Mechanisms of very different nature were proposed for each of the rearrangements. For the formation of the all-*trans* isomer, a classical internal rotation around the C11–C12 *cis* double bond, via a diradical transition state **476**, reminiscent of the transition states characterized in the visual process (see section 4.2), was proposed. The formation of 13-*cis*-retinal would be explained by a cascade of pericyclic reactions, a reversible [1,7]-H sigmatropic shift between the methyl group at C9 and H at C14 to give **477**, followed by a 6- π -oxa-electrocyclic ring closure of the resulting 11,13-di-*cis*-retinal (**478**) and the reverse ring-opening of the corresponding 2*H*-pyran **479** (Scheme 59).

Scheme 60. Biogenetic Route to Carotenoids in *Arabidopsis thaliana*

Other alternatives were also explored for the rearrangement of the pentaenal structure of 11-*cis*-retinal (**5**), including six- and four-electron electrocyclizations as well as their heteroelectrocyclic variants, but their energies were predicted to be much higher. Experiments using 11-*cis*-retinal labeled with deuterium at C19 confirmed the mechanistic proposal and also revealed an unprecedented outcome on the product composition of isotopologues.⁴⁴⁶ The rearrangement of 19,19,19-*d*₃-11-*cis*-

retinal (**5-d**₃) under the same thermal conditions (80 °C in C₆D₆) was considerably slower than that of the protium isotopologue due to the primary isotope effect involved in the sigmatropic shift step. Moreover, the product distribution was inverted in the thermal isomerization of **5-d**₃ when compared to that of **5**, which arises entirely from the primary KIE affecting the [1,7]-H sigmatropic shift.⁴⁴⁶

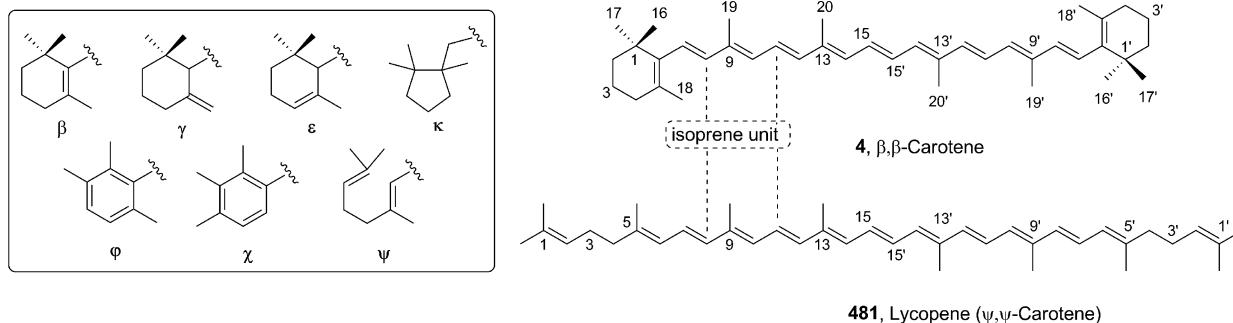


Figure 20. Representative carotenoids and structures of end groups with the common Greek-letter prefixes.

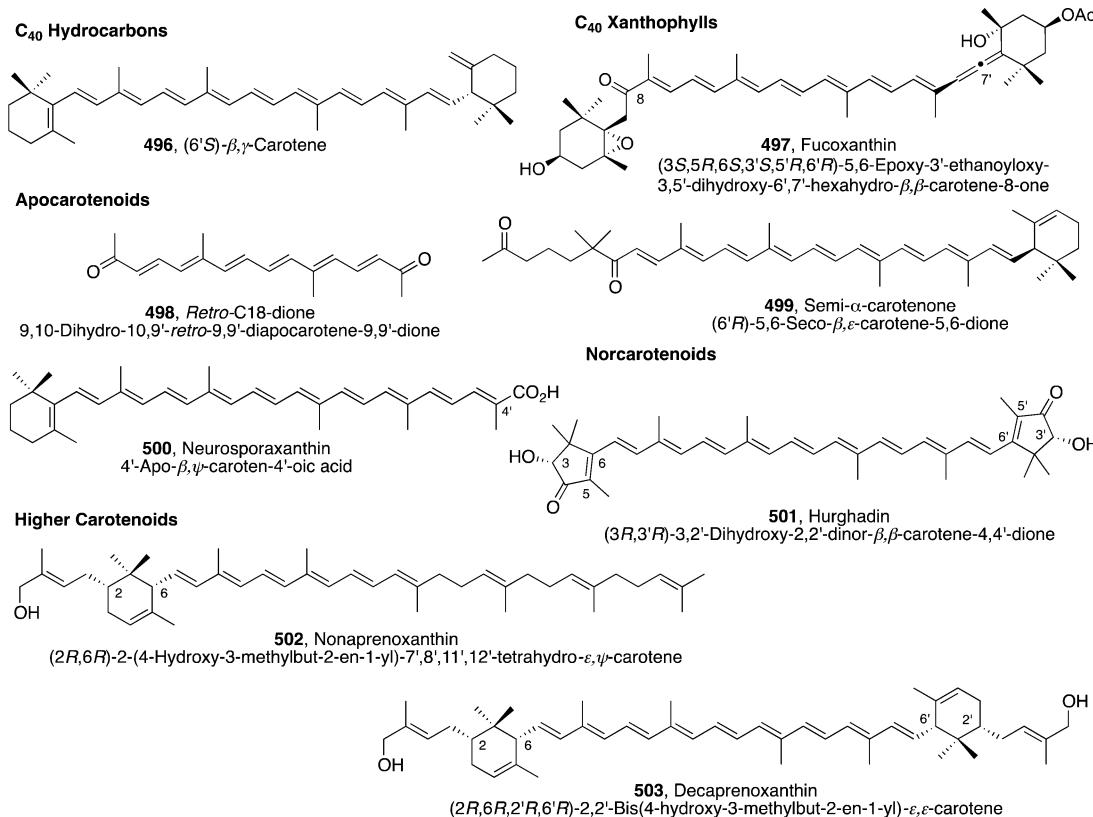


Figure 21. Representative structures of carotenoid divisions.

9. BIOSYNTHESIS OF CAROTENOIDS

The vast majority of the more than 750 carotenoids currently known derive from the desaturation of precursor phytoene (485, a C₄₀ linear tetraterpene) obtained via the mevalonate (482) biosynthetic pathway (Scheme 60).⁴⁴⁷ Two molecules of geranyl geranyl diphosphate (483) undergo the head-to-head dimerization that reverses the connection of the isoprene units at the central region of the carotenoid structure relative to other terpenes. The formation and ring-opening of prephytoene diphosphate (484), a tetrasubstituted cyclopropane derivative,⁴⁴⁷ are assisted in plant carotenoid biosynthesis (the scheme shows the pathways and enzymes identified in *Arabidopsis thaliana*; for other species and complete description of carotenoid formation, see <http://www.genome.jp/kegg/pathway.html>) by the light-responsive enzyme phytoene synthase (PSY) and generate 15-cis-phytoene (485). An ordered sequence of dehydrogenations then provides 9,15,9'-tri-cis- ξ -carotene (486), which isomerizes by the combined

action of ξ -carotene isomerase (Z-ISO) and light to 9,9'-di-cis- ξ -carotene (487). The di-cis isomer is transformed into 7,9,7',9'-tetra-cis-lycopene (488) by the enzyme ξ -carotene desaturase (ZDS). The final conversion to all-trans-lycopene (481) is mediated by carotenoid isomerase (CRTISO) in a process also induced by light.

Lycopene (481) can undergo cyclizations to produce the diversity of terminal end-cyclic carotenoids by the action of cyclases (LCYs). Figure 20 depicts the systematic nomenclature of the end groups of the carotenoid family using Greek-letter prefixes:^{40b} acyclic (ψ), cyclohexene (β, ε), methylenecyclohexane (γ), cyclopentane (κ), and aryl (ϕ, χ), which are used in alphabetical order in the case of nonsymmetrical carotenoids. Only the bifurcations leading to the α - and β -carotene classes and to the corresponding oxygenated compounds (xanthophylls) are shown in Scheme 60. ε -Carotene synthase (ε LCY) operates as a first committed step in this process to provide α -carotene (or β,ε -carotene, 489), and its regulation modulates the ratio of lutein (491), the most abundant carotenoid, to the

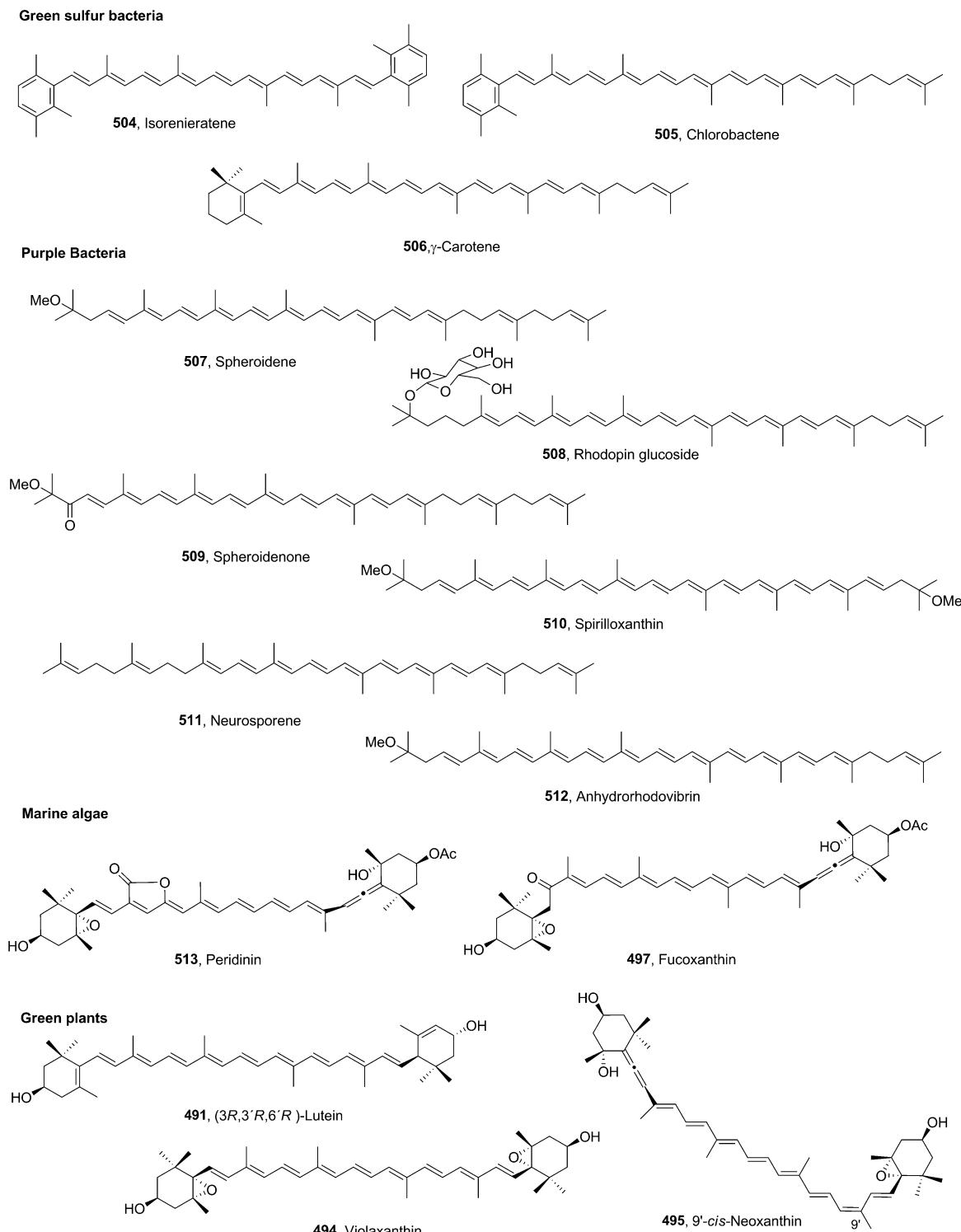


Figure 22. Selected carotenoids of photosynthetic organisms.

members of the β -class, with β,β -carotene (**4**) as the parent structure.^{10b}

Oxidative metabolism to generate the xanthophylls is catalyzed by β -hydroxylases and ϵ -hydroxylases in the case of the α -carotene branch. For the β -branch, epoxidation by zeaxanthin epoxidase (ZEP) converts zeaxanthin (**492**) into violaxanthin (**494**) via the monoepoxide antheraxanthin (**493**). This process is reversible, and the action of violaxanthin de-epoxidase (VDE) returns zeaxanthin (**492**). The so-called

"xanthophyll cycle"⁴⁴⁸ (alternative xanthophylls are involved in similar interconversions in other species) is responsive to light and plays an important role in photoprotection (see section 10.2).

Further modifications of the C₄₀ polyunsaturated backbone by oxidation (hydroxylases, ketolases), rearrangements, and excisions give rise to the different skeletons of the family.

Figure 21 includes the structures of representative members of the carotenoid family with the common names and the

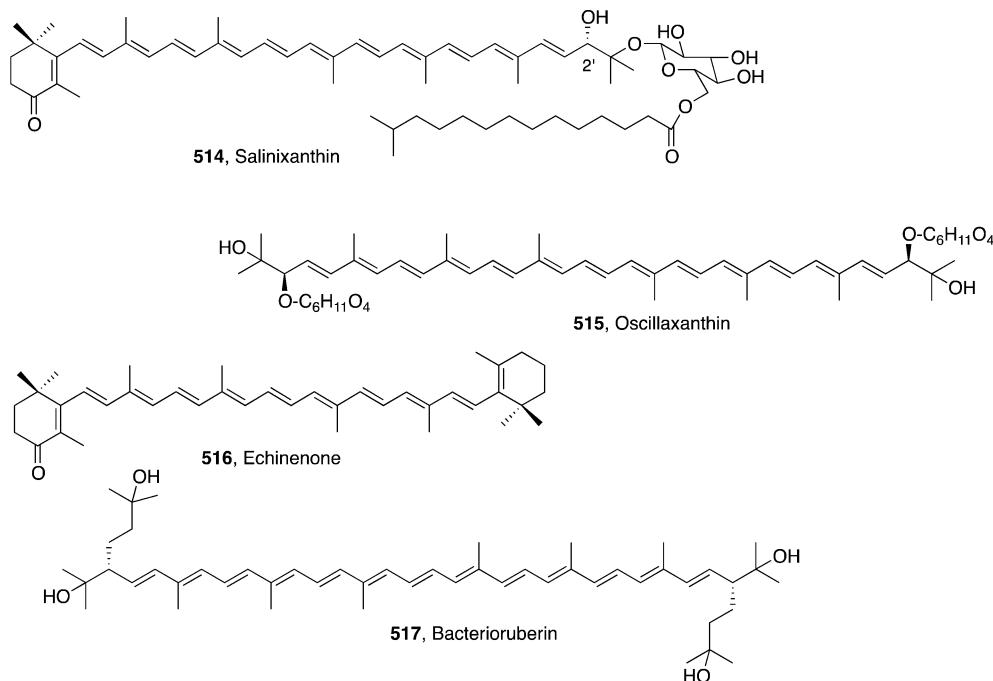


Figure 23. Carotenoids in complexes that contain all-*trans*-retinal (2).

systematic nomenclature. Examples of the regular C₄₀ carotenoids (496), xanthophylls (497), apocarotenoids, secocarotenoids and norcarotenoids (498–501), and higher carotenoids (>C₄₀) produced by certain bacteria (502,503) are included.⁴⁴⁹

10. BIOLOGICAL FUNCTIONS OF CAROTENOIDS

Carotenoids play distinctive and seemingly disparate roles in nature.^{2d,13} They are key components of the light-harvesting antenna complexes (LHCs) of photosynthetic organisms and are involved in photoprotection. These highly conjugated polyenes are mainly responsible for the bright colors ranging from yellow to reddish of the producing organisms and also accumulate in light exposed tissues of feeding animals.^{10b,450} The bright colors of plants and flowers are used as attractants for pollination and seed dispersal and are considered as sexual traits in some species. The antioxidant properties of carotenoids, which are linked to their conjugated chromophores, are considered beneficial to human health. Some natural carotenoids, and their cleavage products, the apocarotenoids and diapocarotenoids, are commercially important in the nutraceutical industry as additives, vitamin supplements, food products, and cosmetics.^{10b} A particular class of apocarotenoids, the retinoids (vitamin A and its derivatives),^{2d} are fundamental substances for cell growth and differentiation in embryo and throughout life.

Like most other animals, humans must acquire carotenoids from the diet. Only some human protist parasites (e.g., *Plasmodium* and *Toxoplasma*),⁴⁵¹ as well as aphids (plant lice)⁴⁵² and the two-spotted spider mite *Tetranychus urticae*,⁴⁵³ have acquired the ability to synthesize carotenoids. Protists produce carotenoids in well-defined subcellular organelles, the plastids, remnants of secondary endosymbiosis of a photosynthetic eukaryote, and might use these polyenes as antioxidants.⁴⁵¹ According to phylogenetic analyses,⁴⁵⁴ carotenoid genes have most likely been horizontally transferred from fungi into the animal's genomes.^{452,453} Whereas in the spider

mite carotenoids are associated with induction of the diapause (a physiological state of dormancy or delay in development under adverse environmental conditions), aphids might have developed an archaic photosynthetic system based on these pigments (see section 10.1.4).⁴⁵⁵

10.1. Photosynthesis

Only about 50 naturally occurring carotenoids (some are shown in Figures 22 and 23 and in Scheme 60) are involved in LHCs of photosynthetic organisms. Carotenoids transfer the sun's energy to acceptor chlorophyll molecules (Chls) to initiate photosynthesis, but also regulate the energy flow protecting the photosynthetic apparatus from photoinduced damage.⁴⁵⁶ The carotenoids are bound to discrete pigment–protein complexes in the proximity of strongly coupled Chls, which are the major pigments of the complexes. Only in the peridinin–chlorophyll complex (PCP)⁴⁵⁷ from the dinoflagellate *Amphidinium carterae* does the carotenoid peridinin (513, Figure 22) outnumber (4:1 ratio) the Chls.⁴⁵⁸

10.1.1. Photophysical Properties of Carotenoids. The S₀ ground state of C₂-symmetric carotenoids is of A_g⁻ symmetry. Because the lowest singlet excited state, S₁ (A_g⁻), is one-photon optically forbidden, the S₂ state (B_u⁺) is the lowest one-photon optically allowed state.⁴⁵⁹ For β,β-carotene (4), the S₀→S₂ transition reaches a maximum at 20 800 cm⁻¹, and the S₁ state is located at roughly 14 500 cm⁻¹. The strong vibronic coupling between the Franck–Condon S₂ and S₁ states of β,β-carotene (4) has been demonstrated by broadband femtosecond optical spectroscopy measurements.⁴⁶⁰ For most carotenoids, the internal conversion (IC) processes from S₂ to S₁ occur in approximately 100 fs, and from S₁ to S₀ in 1–200 ps (depending upon the number of conjugated double bonds).^{459a,461} The dynamics of the S₁ state can be studied without interference from the S₂ (or the “dark state”, located between S₁ and S₂ in certain carotenoids such as spheroidene, 507, Figure 22⁴⁶²) using two-photon excitation with near-infrared light.⁴⁶³

10.1.2. Carotenoids in Light-Harvesting Complexes (LHCs). In higher plants, the thylakoid membranes of chloroplasts, which stack in structures known as grana, carry the photosystems and the LHCs.⁴⁶⁴ Photosystem I (PSI)⁴⁶⁵ is an integral membrane protein complex composed of more than 110 cofactors and arranged into two main subunits, PsaA and PsaB. The P700 reaction centers are formed by two Chls and the associated antennas. Electron flow from the reaction centers is used to shuttle H⁺ across the thylakoid membrane to produce ATP. The released electrons ultimately reduce NADP⁺ to NADPH, the cofactor for transformation of CO₂ into sugars.⁴⁶⁴ Photosystem II (PSII)⁴⁶⁴ is a dimer with two sets of reaction centers (D1 and D2) and their antenna complexes (CD43 and CD47).⁴⁶⁶ The major part of the light-harvesting antenna are monomeric (minor LHCII) and trimeric (major LHCII) pigment–protein complexes.

In view of the short lifetimes of the S₂ and S₁ states of carotenoids as compared to those of other naturally occurring pigments, and the forbidden nature of the S₀ → S₁ transition that results in a negligible transition dipole moment, the evolutionary selection of these natural products as components of LHCs is most remarkable.^{456,459b}

Six peripheral antenna proteins in PSII (LhcB1–6) and four in PSI (LhcA1–4) have been characterized. The X-ray structure of trimeric LHCII (LhcB1–3 monomers) shows binding sites for 14 Chls (8 Chl *a* and 6 Chl *b*) and 4 xanthophylls (L1 and L2 for lutein 491, N1 for 9'-*cis*-neoxanthin 495, and V1 for an undetermined violaxanthin/zeaxanthin xanthophyll cycle pigment).⁴⁶⁷ Sequence similarities predict similar structures for other antenna proteins despite their differences with respect to oligomerization properties and to the number of Chl units and xanthophyll binding sites that these complexes show. Common to all structures is the lutein binding site at L1.⁴⁴⁸

Carotenoids bound to LHCs absorb sunlight maximally in the blue-green visible range ($\lambda_{\text{max}} \approx 450\text{--}550\text{ nm}$), and transfer this excitation energy to nearby (bacterio)chlorophylls, (B)Chls, which absorb in the red region ($\lambda_{\text{max}} \approx 800\text{--}850\text{ nm}$). The process involves singlet–singlet excitation-energy transfer (EET) from carotenoids to (B)Chl. (B)Chl has two distinct absorption bands (designated as Q_x and Q_y, the highest and lowest excited states, respectively) due to $\pi \rightarrow \pi^*$ transitions in the visible to infrared region. The S₂-mediated carotenoid-to-(B)Chl energy transfer occurs in 100–300 fs via the Förster mechanism, and operates in almost all carotenoid-containing LHCs. Singlet excitation energy of lutein (491, Scheme 60) is transferred exclusively to Chl *a* and not to Chl *b*, whereas 9'-*cis*-neoxanthin (495, Scheme 60) was found to transfer its energy mostly toward Chl *b*. The efficient energy transfer from the carotenoid donor to the (B)Chl acceptor is favored by the optimal distance (between 3 and 10 Å) and the appropriate orientation of these chromophores, as revealed by the crystal structures of LHCs.⁴⁵⁶

The S₁ state only acts as efficient energy donor in the case of carotenoids with shorter conjugated chromophores and in complexes that contain Chl *a* as acceptor.^{459a} In purple-bacteria LHCs, ultrafast spectroscopic measurements have shown that singlet–singlet EET involves the S₂ → Q_x and S₁ → Q_y pathways. For example, energy transfer from the S₁ state of spheroidene (507, Figure 22) to BChl has been measured in the LH2 complex of *Rhodobacter sphaeroides*. In contrast, the energy of the S₁ state of the more conjugated rhodopin glucoside (508, Figure 22), which occurs in concentric rings with BChl *a* within a protein matrix in the LH2 complex of the purple bacteria

Rhodopseudomonas acidophila,⁴⁶⁸ is too low for efficient S₁-mediated energy transfer, and therefore the S₂ state becomes the main energy donor.⁴⁶⁹ About 50% of the light harvested by the carotenoid (at $\lambda_{\text{max}} \approx 450\text{--}550\text{ nm}$) is transferred from the S₂ state to the acceptor BChl *a* (B850 Q_y, the lowest singlet excited state in the complex), and the rest is dissipated through internal conversion (IC) processes.⁴⁶¹ The nonradiative decay of S₁ to the ground state in this system occurs in about 2 ps.⁴⁶¹

A reverse singlet–singlet EET from BChl to the carotenoid spirilloxanthin (510, Figure 22, which has 13 conjugated double bonds) has been characterized in the photosynthetic core–antenna complexes of the purple bacteria *Rhodospirillum rubrum* S1. By this process, a large amount (40%) of the excitation energy transferred by S₂ of spirilloxanthin (510) to the Q_x state of BChl (with 43% efficiency) is returned to the carotenoid, which acts as an efficient quencher of the BChl Q_x excited state.⁴⁷⁰ This reverse singlet–singlet EET from Q_x of Chl to S₂ of carotenoids has interesting parallels in the nonphotochemical quenching (NPQ) involving LHCII of higher plants⁴⁷¹ and has been reproduced in artificial light-harvesting dyads.⁴⁷²

10.1.3. Retinoid/Carotenoid Complexes. (See Section 4.1) Xanthorhodopsin (section 4.1)¹⁵³ from *Salinibacter ruber* contains sanilixanthin (514, Figure 23), a glycosylated carotenoid acylated with a fatty acid,¹⁵⁴ and all-trans-retinal (2) in a molar ratio of about 1:1. The two chromophores strongly interact to extend the absorption of the bacterium to the green and blue spectral regions. Sanilixanthin functions as a light capture antenna, which transfers energy from its S₂ state to S₁ of the retinal chromophore for uphill transmembrane proton transport.¹⁵⁶ The X-ray structure of xanthorhodopsin shows a center-to-center distance between both chromophores of ca. 11 Å with an orientation of their molecular axes of 46°, which explains the highly efficient (between 30% and 50%) energy transfer measured in the complex.¹⁵⁵ This natural model of an EET device is the simplest photosynthetic protein complex so far discovered that features a separate light-harvesting function. As such, it is the subject of intense theoretical⁴⁷³ and experimental investigations⁴⁷⁴ aimed to understand the effects of the pseudo-Coulombic interactions between donor and acceptor chromophores in the photochemical and photophysical properties of the natural EET device.⁴⁷⁴

Gloeobacter violaceus rhodopsin can be reconstituted into *E. coli* with bound salinixanthin (514). This carotenoid adopts a conformation similar to that found in xanthorhodopsin, and functions as a light-harvesting antenna to transfer energy to retinal.⁴⁷⁵ This might be the function of β,β-carotene (4), oscillaxanthin (oscillol diglycoside, 515), and echinenone (516, Figure 23), the three natural carotenoids found in cultures of *G. violaceus*.

In the trimeric retinal protein–carotenoid complex archaerhodopsin-2 (aR2, section 4.1) from *Halorubrum sp. aus-2*¹⁵⁷ and in the trimeric structure of halorhodopsin (HR, section 4.1) from *Natronomonas pharaonis*, the carotenoid bacterioruberin (517, Figure 23) binds a crevice between the subunits of the trimer,^{158,159} increasing the thermal stability of these complexes.

10.1.4. Photosynthesis in Animals? The exciting discovery that aphids synthesize carotenoids has led one to question the role of the pigments in the animal's physiology. The pigments form a layer between 0–40 μm deep under the insect's cuticle. In addition to provide pigmentation and antioxidant protection, it has been shown that the aphid

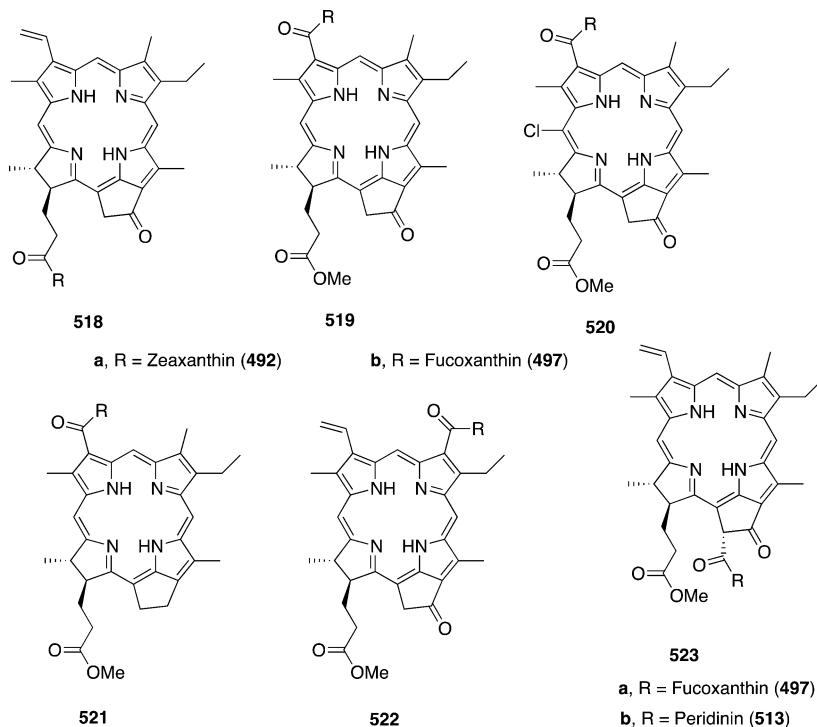


Figure 24. Carotenoid–pyropheophorbide dyads.

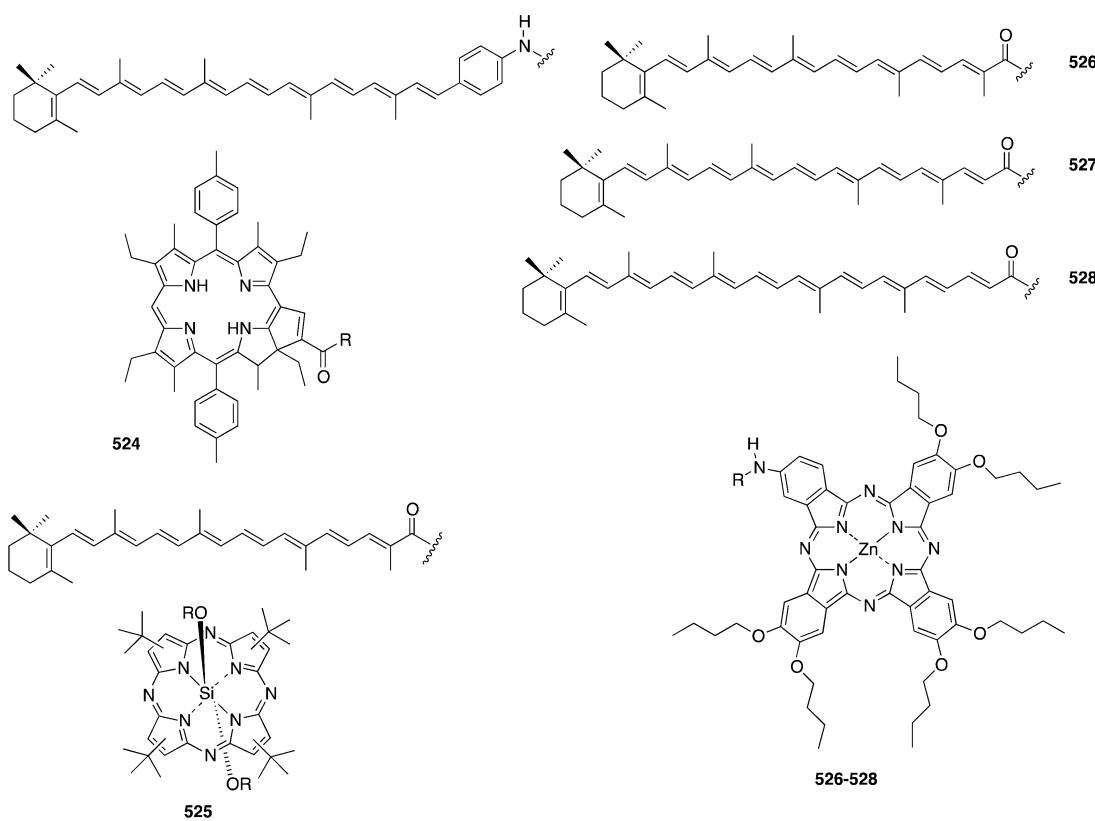


Figure 25. Carotenoid–porphyrin dyads.

carotenoids are involved in ATP production,⁴⁵⁵ and that the amount of ATP produced depends upon the level of carotenoids: green aphids are more productive than white ones, which are almost devoid of these pigments; orange insects, which produce intermediate amounts of carotenoids,

generate more ATP when placed in the light.⁴⁵⁵ If the function of this archaic photosynthetic system is confirmed, it would be the first example of photoheterotrophy in animals.

10.1.5. Artificial Photosynthetic Devices. The natural LHCs have been an inspiration for the design of synthetic

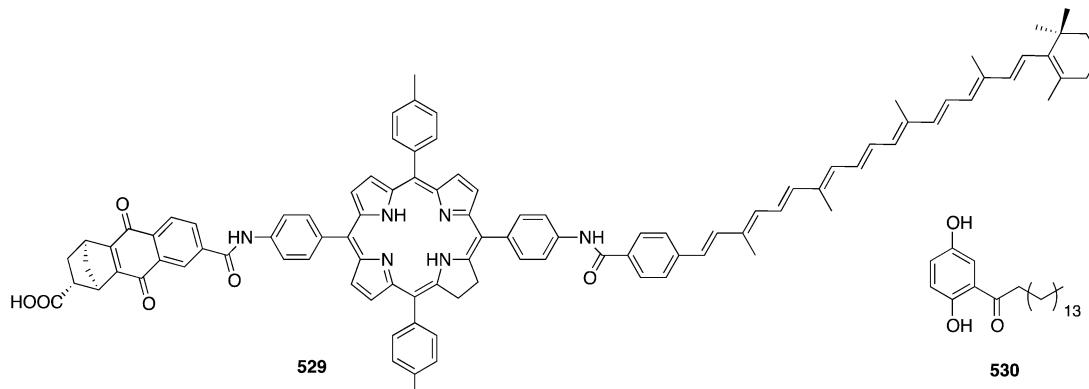


Figure 26. A carotenoid–porphyrin–naphthoquinone triad for light-driven transmembrane Ca^{2+} transport.

antennae, and it has been proposed that emulating their efficiency can be further developed into artificial photosynthetic systems and photochemical fuel production devices.⁴⁷⁶

Carotenoid–pyropheophorbide dyads (**518–522**, Figure 24) with covalently bound (through their secondary alcohol) zeaxanthin (**492**) or fucoxanthin (**497**) as donors were found to qualitatively reproduce well the natural system, according to femtosecond transient absorption spectroscopy and steady-state fluorescence excitation spectroscopy measurements.⁴⁷⁷ For fucoxanthin–pyropheophorbide dyads, energy transfer was found to occur from the higher-lying fucoxanthin S_1 state (which is of higher energy than the corresponding S_1 state in zeaxanthin) to the lower-lying pyropheophorbide S_1 (Q_y) state and with 12–44% efficiency depending upon the mutual orientation of the chromophores. No evidence of energy transfer from the zeaxanthin S_1 state to the pyropheophorbide S_1 (Q_y) state was found in the five zeaxanthin-containing compounds (**518–522a**), but instead efficiencies of up to 15% were measured for transfer from the S_2 state.⁴⁷⁷ Connecting fucoxanthin (**497**, Figure 21) and peridinin (**513**, Figure 22) through their secondary alcohols to a different position of the pyropheophorbide scaffold generated the dyads **523**.^{478,457} Peridinin was found to transfer energy to pyropheophorbide S_1 (Q_y) from its S_1/ICT (intramolecular charge transfer) state with 80% efficiency in benzene, which is similar to that found in the natural PCP of *A. carterae*.⁴⁵⁸ Solvent polarity appears to be important for tuning the energy transfer efficiency in these constructs, because it decreased to 20% in acetonitrile.^{457,478}

An aniline with a *p*-undecaene carotenoid bound through an amide linker to a 5,15-diaryloctaalkylporphyrin (**524**, Figure 25) transferred energy with an efficiency greater than 70%, apparently from a S_2 state as the sole donor.⁴⁷⁹ Shorter carotenoids attached to the apical position of a central silicon atom of a Si-phthalocyanine molecular triad (**525**) showed an inefficient (below 70%) S_2 channel but a highly efficient (nearly 90%) S_1 channel.⁴⁸⁰ Carotenoids with 10 and 11 double bonds in carotenoid–phthalocyanine dyads (**526–528**)⁴⁸¹ quenched the excited S_1 state of phthalocyanine and dissipated the excess absorbed energy, thus mimicking the critical photoprotective function of carotenoids in higher plants (vide infra).⁴⁷²

Other carotenoid–porphyrin–naphthoquinone triads mimicked light-induced biological phenomena such as photodriven charge separation,⁴⁸² conversion of light energy into proton potential,⁴⁸³ light-driven production of ATP catalyzed by F_0F_1 -ATP synthase in an artificial photosynthetic membrane using a lipid soluble quinone shuttle,⁴⁸⁴ and a light-driven transmembrane Ca^{2+} pump using a redox-sensitive, lipophilic Ca^{2+} -

binding shuttle molecule.⁴⁸⁵ In the latter case, the construct **529** (Figure 26) functioned as an intramembrane artificial photosynthetic reaction center to drive calcium ions across the bilayer of a liposome generating both a calcium ion concentration gradient and a membrane potential.⁴⁸⁵ The lipophilic Ca^{2+} -binding 2,5-dihydroxyacetophenone derivative **530** acting as shuttle diffuses across the membrane and is oxidized by the carotenoid radical cation formed by photoinduced electron transfer of the photoactive triad asymmetrically disposed across the lipid bilayer. After release of Ca^{2+} to the internal compartment, the quinone diffuses back and gets reduced by the radical anion of the triad.⁴⁸⁵

10.2. Carotenoids in Photoprotection

10.2.1. Photoprotection of Photosynthetic Organisms. LHCs of photosynthetic organisms have evolved to allow optimal photosynthesis at low light intensities.⁴⁸⁶ Excess light leads to the formation of reactive oxygen species (ROS),⁴⁸⁷ which may damage the photosynthetic machineries. Two major pathways for ROS formation in chloroplasts are known:⁴⁴⁸ (1) the electron transfer to O_2 at the acceptor side in PSI and PSII, generating superoxide radical anion and its downstream products hydrogen peroxide or hydroxyl radicals; and (2) the energy transfer from triplet chlorophyll ($^3\text{Chl}^*$) to O_2 , which forms singlet oxygen ($^1\text{O}_2^*$).⁴⁸⁸ Genetic programs activated by the release of $^1\text{O}_2^*$ also contribute to plant damage.⁴⁸⁹

In the PSII reaction center, $^1\text{O}_2^*$ deactivation is provided by β,β -carotene. Other carotenoids deactivate $^1\text{O}_2^*$ and $^3\text{Chl}^*$, and reduce ROS formation by nonphotochemical fluorescence quenching (NPQ), a mechanism for thermal dissipation of excess light energy at the level of $^1\text{Chl}^*$.⁴⁹⁰ Xanthophylls are the main carotenoids implicated in NPQ of LHCs through changes in their oxidation state (xanthophyll cycles).⁴⁴⁸ The name “xanthophyll cycle” describes the reversible interconversion of certain xanthophylls by epoxidation/de-epoxidation reactions occurring selectively at the tetrasubstituted olefin of the cyclohexene ring(s). PSII uses these cycles to switch from a light-harvesting state in low light or darkness (with epoxidized xanthophylls and shorter polyenes) to an energy-dissipating state (with nonepoxidized xanthophylls having longer polyenes) under high light conditions, and through this mechanism plants adapt to changes in light intensities. Two xanthophyll cycles have been characterized in land plants: the violaxanthin cycle (violaxanthin **494** to zeaxanthin **492** via monoepoxide antheraxanthin **493**, see Scheme 60) in all plants and the lutein epoxide cycle (lutein **491** to lutein epoxide **533**, Figure 27),

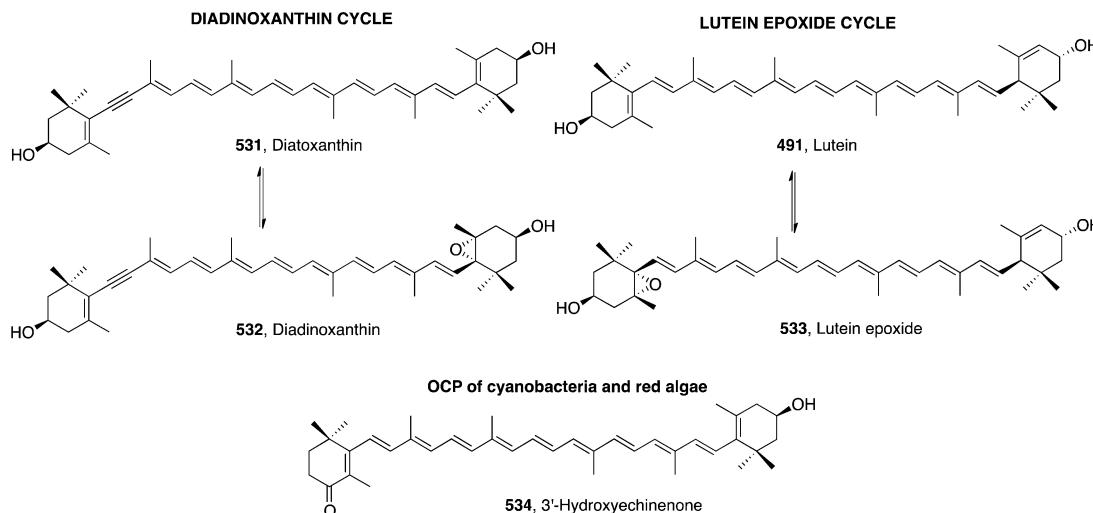


Figure 27. Additional xanthophyll cycles (for the violaxanthin cycle, see Scheme 60) and other carotenoids involved in photoprotection.

only in some species. Changes of conformations of *9'-cis*-neoxanthin (**495**) and lutein (**491**) have been measured by resonance Raman spectroscopy when the major LHCII complex switches to the energy dissipative state.⁴⁹¹ Lutein is the most abundant xanthophyll in higher plants and, in addition to its role in stabilizing LHCs and harvesting light, contributes to protoprotection by deactivating $^3\text{Chl}^*$. Zeaxanthin, on the other hand, is essential in energy dissipation and deactivation of $^1\text{Chl}^*$. The involvement of this xanthophyll in NPQ is consistent with the overexpression of β -carotene hydroxylase observed in *A. thaliana* under stress conditions, which produces an increase in zeaxanthin (**492**) content.⁴⁹² Xanthophylls also modulate LHCII membrane protein function⁴⁹³ and prevent the oxidative damage of membrane lipids under high light stress conditions due to their antioxidant properties.⁴⁹²

NPQ depends on light-regulated and xanthophyll-dependent processes that are mediated by specific proteins, which, after sensing excess generation of chemical energy and increased lumen pH, induce a conformational change to LHC complexes.^{448,494} Depending upon the phyla, different NPQ mechanisms have been characterized.

In plants and algae, NPQ is controlled by the pH sensing protein LHCSR or PsbS (a subunit of PSII),⁴⁹⁵ which transfers the pH-induced signal to PSII antenna proteins, and by the formation of zeaxanthin (**492**).^{486,490} Picosecond Chl fluorescence spectroscopy of intact leaves confirmed the intervention of two different quenching sites, the PsbS-dependent in the major LHCII and the zeaxanthin-dependent in the remaining minor antenna of PSII.⁴⁹⁶ Both the dissociation of PsbS from its dimeric structure and the formation of zeaxanthin (**492**) from violaxanthin (**494**) are induced by low pH, and thus when luminal pH is raised the process returns to normal photosynthesis.⁴⁹⁷ In *A. thaliana* lacking zeaxanthin (**492**), the proportion of NPQ remaining was ascribed to a subpopulation of zeaxanthin molecules formed during photosynthetic electron transport conditions.⁴⁹⁸ Two-photon excitation of the S_1 state in LCHII of *A. thaliana* led to the characterization of quenching excitonic Car S_1 –Chl states. In vivo, these short-living Car–Chl states due to excitonic Car S_1 –Chl αQ_y interactions during high illumination would serve as traps and dissipation valves for excess excitation energy. The formation of the excitonic states, which correlates linearly with NPQ, depends on the presence of PsbS and

zeaxanthin (**492**).⁴⁹⁹ A red-shifted zeaxanthin-dependent optical signal in the carotenoid peak of the triplet-minus-singlet spectrum of *A. thaliana* leaves and pigment-binding proteins, characterized by time-resolved differential spectroscopy *in vivo*, was found to correspond to the LhcB4–6 antenna components of PSII and the LhcA1–4 subunits of PSI. The signal strongly correlated with photoprotection in the chloroplasts. Fluorescence-detected magnetic resonance analysis showed a decrease in signals assigned to $^3\text{Chl}^*$ of LhcB4–6 upon zeaxanthin binding, which confirmed the increased efficiency of this xanthophyll in controlling $^3\text{Chl}^*$ formation and preventing the production of $^1\text{O}_2^*$. Moreover, the zeaxanthin-dependent photoprotection remains fully active in mutant *npq4* lacking PsbS.⁵⁰⁰

In green alga, the photoprotection strategy is controlled by the ΔpH and depends not only on xanthophylls but also on a specific antenna protein LhcBm1.⁵⁰¹ In brown algae and diatoms, NPQ is mediated by the LHCSR protein, but the conversion of violaxanthin (**494**) to zeaxanthin (**492**) in the violaxanthin cycle and of diadinoxanthin (**532**) into diatoxanthin (**531**, Figure 27) in the diadinoxanthin cycle are also required.⁵⁰² In cyanobacteria and red algae, NPQ⁵⁰³ is triggered by conformational changes of the photosensor water-soluble orange carotenoid protein (OCP),⁵⁰⁴ which contains 3'-hydroxyechinenone (**534**, Figure 27) as the chromophore.⁵⁰⁵

NPQ operates on a time-scale of seconds to minutes, and it has been proposed to occur by a charge-transfer mechanism involving a carotenoid radical cation⁵⁰⁶ and/or by Chl-to-carotenoid energy transfer.⁴⁴⁸ An alternative interpretation for the quenching process, obtained from studies of LHCII oligomers, suggests a Chl-to-Chl charge-transfer state as intermediate, without the involvement of energy transfer to carotenoids.⁵⁰⁷

A radical cation of the carotenoid spheroidene (**507**, Figure 22) has been spectroscopically characterized by a spectral band at $\lambda_{\text{max}} \approx 960$ nm, which decays within 8 ps,⁴⁶⁹ after rapid formation (~ 200 fs) in the LH2 complex of *Rhodobacter sphaeroides*. The formation of a zeaxanthin radical cation was proposed by femtosecond transient Chl absorption experiments on thylakoid membranes under conditions of maximum, steady-state feedback deexcitation. The mechanism of formation involves the ultrafast charge separation of a Chl–zeaxanthin heterodimer, which undergoes excitation transfer from

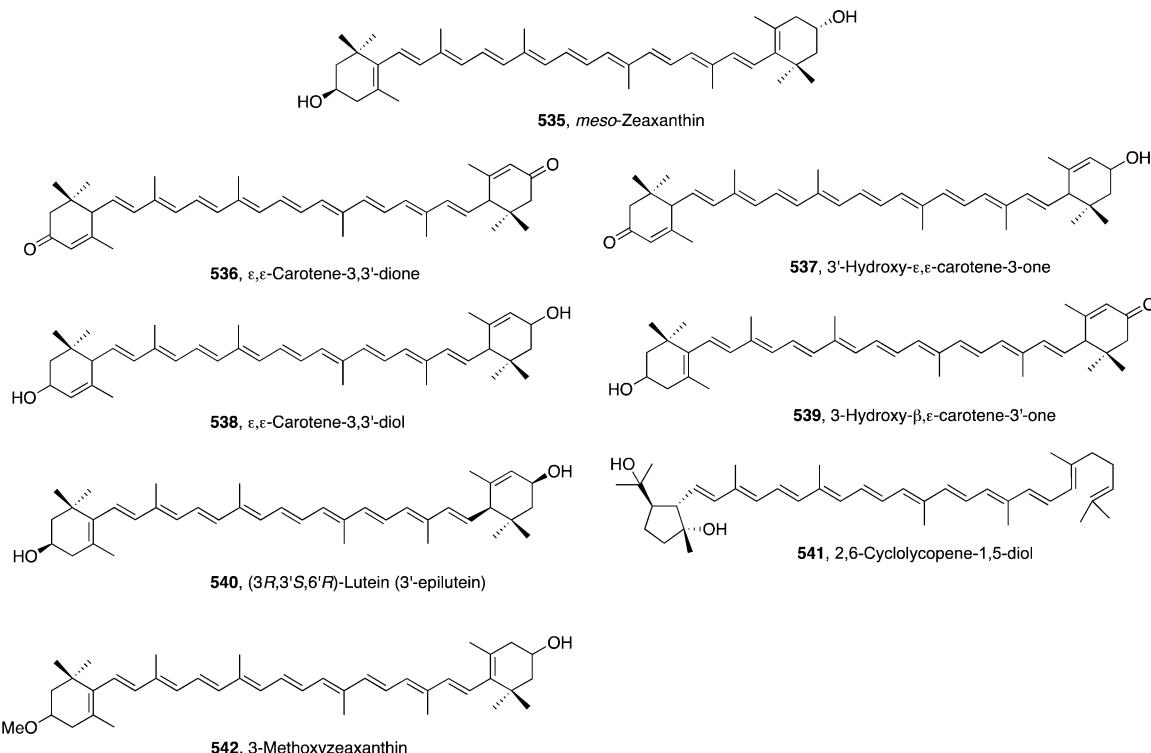


Figure 28. Xanthophylls of the macula of humans and monkeys and some of the oxidation products (the absolute configuration of the compounds is unknown, with the exception of that shown for **540**).

$^1\text{Chl}^*$.^{506a} Zeaxanthin radical cation formation was found by near-infrared transient absorption spectroscopy to occur solely in minor LHCs.⁵⁰⁸

To clarify the mechanism of $^3\text{Chl}^*$ quenching by carotenoids, the triplet absorption spectra of a series of carotenoids ranging from $N = 9$ to 13 conjugated double bonds (neurosporene **511**, spheroidene **507**, lycopene **481**, anhydrorhodovibrin **512**, and spirilloxanthin **510**, see Figure 22) were recorded in solution and compared to those of the reconstitution complexes (LHII, LHI, RC, or RC-LHI).⁵⁰⁹ T_1 lifetimes in solution were found to decrease with N . The twisting of the carotenoid chain on going from solution to LHCII was considered an important contributor to the shifting to shorter lifetimes, but other structural factors that affect the triplet absorption spectra and the lifetimes of the reconstituted complexes also contribute.⁵⁰⁹ DFT and TDDFT studies of the ground state and first excited state of xanthophylls (violaxanthin **494**, zeaxanthin **492**, and lutein **491**) suggested that the *s-cis* conformations are biologically relevant for light harvesting and photoprotection.⁵¹⁰ Other studies concluded that triplet states of carotenoids in LHCs are generated without conformational changes with respect to their structures in solution. In purple bacteria, triplet-triplet transfer from (B)Chl to carotenoids takes place in the nanosecond range. In higher plants LHCs, the triplet wave function is shared between the carotenoid and adjacent Chls, which explains the faster triplet-triplet transfer times in these complexes and the absence of detectable $^3\text{Chl}^*$ upon excitation.⁵¹¹

A recent report has identified a bilin chromophore with fluorescence maximum at $\lambda_{\text{max}} \approx 660$ nm (termed APC $^Q_{660}$) in the core of the phycobilisome, which quenches the excitation of the OCP complex in less than 1 ps and prevents it from reaching PSI and PSII.⁵¹² It was proposed that the blue-green-induced NPQ mechanism in cyanobacteria involves charge

transfer between APC $^Q_{660}$ and the activated 3'-hydroxyechinenone chromophore of OCP,⁵¹² in parallel to the plants NPQ mechanism between the Chl *a*-zeaxanthin coupled excited pair.

10.2.2. Eye Photoprotection. Xanthophyll photoprotection of photosynthetic organisms is paralleled in the human eye, where these compounds are considered to contribute to reduce the risk of developing AMD⁵¹³ and cataracts.⁵¹⁴ About 50% of the total amount of xanthophylls in the retina is concentrated in the *macula lutea*. At the center of the macula (the fovea), where the xanthophyll content is about 1000 times higher than in other tissues, the zeaxanthin/lutein ratio is 2:1 (lutein instead dominates in the peripheral part of the retina). Zeaxanthin is present as a 50:50 mixture of zeaxanthin (**492**) and its stereoisomer *meso*-zeaxanthin (**535**, Figure 28), which must form metabolically, because it is unknown as a natural product. An ocular xanthophyll-binding protein (XBP), member of the carotenoid-binding protein (CBP) family, was isolated from human macula and retina⁵¹⁵ and identified as the π isoform of human glutathione S-transferase (GSTP1),⁵¹⁶ a phase II detoxification enzyme. GSTP1 binds zeaxanthin (**492**) and *meso*-zeaxanthin (**535**) with high affinity and specificity, but not lutein (**491**). The lutein-binding protein in the primate *macula lutea* is StARD3, a member of the steroidogenic acute regulatory domain family (StRD), which also includes silkworm CBP (see section 12).⁵¹⁷

The exact mechanism by which these carotenoids provide eye photoprotection is currently unknown, but these macular pigments (zeaxanthin, lutein, and *meso*-zeaxanthin) might absorb relatively high-energy short-wavelength light and provide protection against oxidative damage.⁵¹⁸ By the first mechanism, xanthophylls can neutralize triplet-state photosensitizers, such as the bis-pyridinium retinoid pigments and the retinal dimers (Figure 9), which absorb light in the short wavelength region ($\lambda_{\text{max}} \approx 450$ nm). Moreover, the polyene

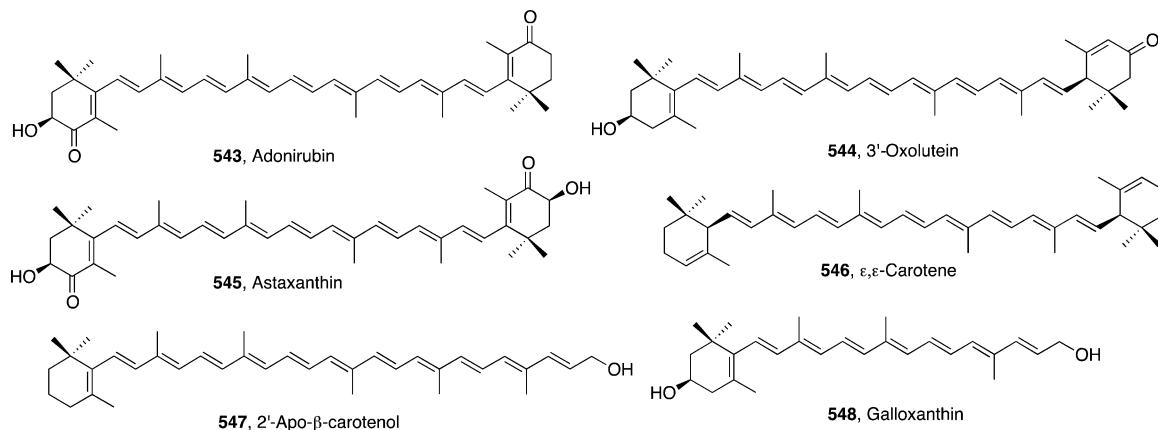


Figure 29. Additional carotenoids isolated from the macula of the Japanese quail.

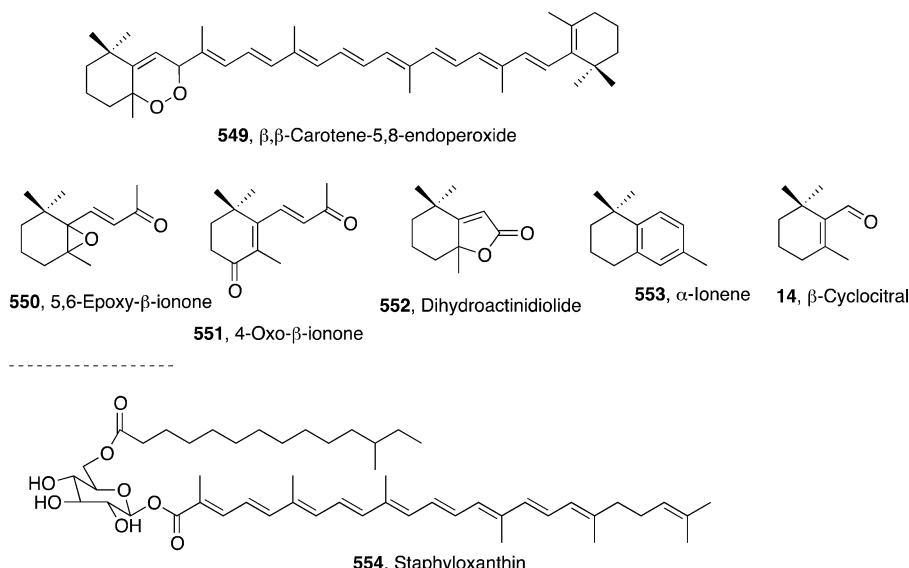


Figure 30. Photooxidation products of β,β -carotene (4), and structure of antioxidant staphyloxanthin (554).

structure of eye xanthophylls might contribute to neutralize singlet oxygen and free radicals, and thus limit the damage to lipid membranes by peroxidation. In this regard, recent studies using EPR to measure $^1\text{O}_2$ formation in eye tissues (post-mortem human macula and retinal pigment epithelium(RPE)/choroid) found that $^1\text{O}_2$ formation was only detected in human RPE/choroid but not in human retina.⁵¹⁹ Because the majority of macular carotenoids is localized to the inner retina far away from the RPE layer, these compounds are unlikely to quench $^1\text{O}_2$, but they can still act as intrinsic filters of short wavelength light to prevent or reduce the generation of $^1\text{O}_2$ in RPE. Moreover, experiments in solution have shown the synergy of the eye xanthophylls, because a mixture of *meso*-zeaxanthin, zeaxanthin, and lutein in 1:1:1 ratio (as in the fovea) can quench more $^1\text{O}_2$ than the individual carotenoids at the same total concentration.⁵¹⁹

Oxidation products of eye xanthophylls have been identified in human and monkey retinas (Figure 28). In addition to a major carotenoid resulting from the oxidation of lutein, 3-hydroxy- β,ϵ -caroten-3'-one (539), several other minor carotenoids (ϵ,ϵ -carotene-3,3'-dione 536, 3'-hydroxy- ϵ,ϵ -caroten-3-one 537, ϵ,ϵ -carotene-3,3'-diol 538, 3'-epilutein 540, and 2,6-cyclolycopene-1,5-diol 541) and double-bond isomers of lutein and zeaxanthin were characterized.⁵²⁰ 3-Methoxyzeaxanthin

(542) was recently identified in human macula but not in peripheral retina or in nonretinal tissues, and its concentration was found to increase with age.⁵²¹

Oxidized carotenoids have also been identified in the retina of the Japanese quail *Coturnix japonica* (Figure 29),⁵²² including adonirubin (543), 3'-oxolutein (544), astaxanthin (545), ϵ,ϵ -carotene (546), 2'-apo- β -carotenol (547), galloxanthin (548), and the three eye xanthophylls. Using labeled dietary xanthophylls, it was shown that lutein (491) generates *meso*-zeaxanthin (535), whereas zeaxanthin (492) must likely be the metabolic precursor of other (apo)carotenoids.⁵²²

10.3. Oxidation of Carotenoids with Reactive Species

10.3.1. Reactions with ROS. Previous studies had identified a series of β,β -carotene (4) oxidation products that are formed by the action of $^1\text{O}_2$ generated upon photo-sensitized oxidation with Rose Bengal, including β -ionone (10), 14'-apo-, 10'-apo-, and 8'-apo- β -carotenals (19, 13, and 9, respectively; Scheme 2), and a 5,8-endoperoxide 549 (Figure 30).⁵²³ More recently, it was demonstrated that light stress induces carotenoid oxidation in *Arabidopsis* leading to the accumulation of volatile derivatives,^{523b} especially β -cyclocitral (14), dihydroactinidiolide (552), and to a lesser extent β -ionone (10). Moreover, β -cyclocitral (14) induced changes in

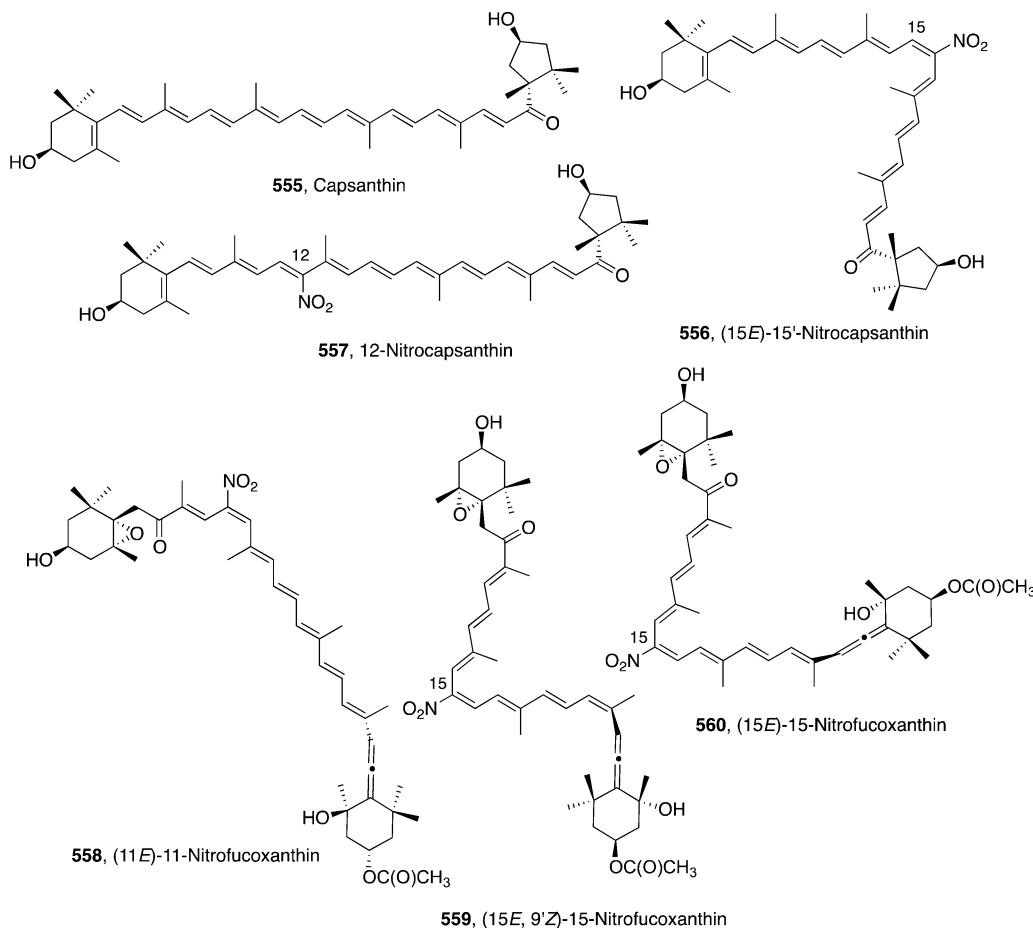


Figure 31. Products resulting from the treatment of capsanthin (**555**) and fucoxanthin (**497**, Figure 21) with peroxinitrite.

gene expression of ${}^1\text{O}_2$ sensitive genes (but not of H_2O_2 gene markers). Thus, $\text{C}_{10}\beta$ -cyclocitral (**14**) may be considered as a stress signal and a messenger involved in sensing and signaling of oxidative stress conditions in plants. Whereas most of these oxidation products with apocarotenoid structures could be formed by CCEs (section 3) and therefore are not specific to ${}^1\text{O}_2$ damage, this is unlikely the case of β,β -carotene-5,8-endoperoxide (**549**, Figure 30), which was found to accumulate rapidly in *Arabidopsis* during high-light stress, and this accumulation was correlated with the extent of PSII photo-inhibition and the expression of various ${}^1\text{O}_2$ marker genes.⁵²⁴ Given that similar endoperoxides formed in vitro by oxidation of xanthophylls lutein (**491**) and zeaxanthin (**492**) were not detected in the plant, the PSII reaction centers and not the antennae could be the major source of ${}^1\text{O}_2$. Thus, the appearance of β,β -carotene-5,8-endoperoxide (**549**) may be used as marker for early detection of stress in plants.

Some of the harmful effects of carotenoids, especially for smokers, as revealed in certain clinical efficacy trials (section 13) might be due to carotenoid-cleavage products originated in oxidation reactions. The oxidation mediated by hypochlorous acid is a model for degradation of carotenoids by oxidants released by polymorphonuclear leucocytes (PMLs).⁵²⁵ GC-MS analysis of the degradation mixture obtained from the treatment of β,β -carotene (**4**) with NaOCl/HOCl in MeOH solution identified the volatile compounds β -ionone (**10**), its 5,6-epoxide and 4-oxo derivatives (**550** and **551**), α -ionene (**553**), and dihydroactinidiolide (**552**, Figure 30). In aqueous solution, the degradation rate was slower, and in addition the

main products were apocarotenals (8'-apo- β -, 12'-apo- β -, and 15'-apo- β -carotenal-retinal; **13**, **16**, and **2**, Schemes 1 and 2). These apocarotenals together with β -ionone (**10**), its 5,6-epoxide (**550**), α -ionene (**553**), and dihydroactinidiolide (**552**) were identified among the large number of substances (between 63 and 103) detected in culture media of activated PMLs.⁵²⁵

Quenching of ROS such as superoxide, peroxide, and hypochlorite produced by the white cells of the organism to combat infection during the inflammatory response acts as a virulence factor for *Staphylococcus aureus*. The polyene chain of the C_{30} carotenoid staphyloxanthin (**554**, Figure 30), identified in *S. aureus*, is partly responsible for ROS quenching (and for the golden appearance of the microorganism).⁵²⁶ Interestingly, because an early step in staphyloxanthin biosynthesis in *S. aureus*, catalyzed by dehydrosqualene synthase (CrtM), is similar in cholesterol biosynthesis, the treatment with a cholesterol-lowering compound resulted in colorless bacteria in vitro, which showed increased susceptibility to killing by human blood in a mouse infection model.⁵²⁷ This is an interesting novel strategy to block infection virulence based on targets discovered by biogenetic reasoning.⁵²⁸

10.3.2. Reactions with Reactive Nitrogen Species.

Reactive nitrogen species are also formed in vivo. Carotenoids undergo fast autoxidation reactions induced by cigarette smoke. 4-Nitro- β,β -carotene (all-*trans* and two mono-*cis* isomers) is the main oxidation product of β,β -carotene (**4**), and possibly forms by smoke-borne reactive nitrogen species. Several apo- β -carotenoids and the 5,6- and 5,8-epoxides were also detected

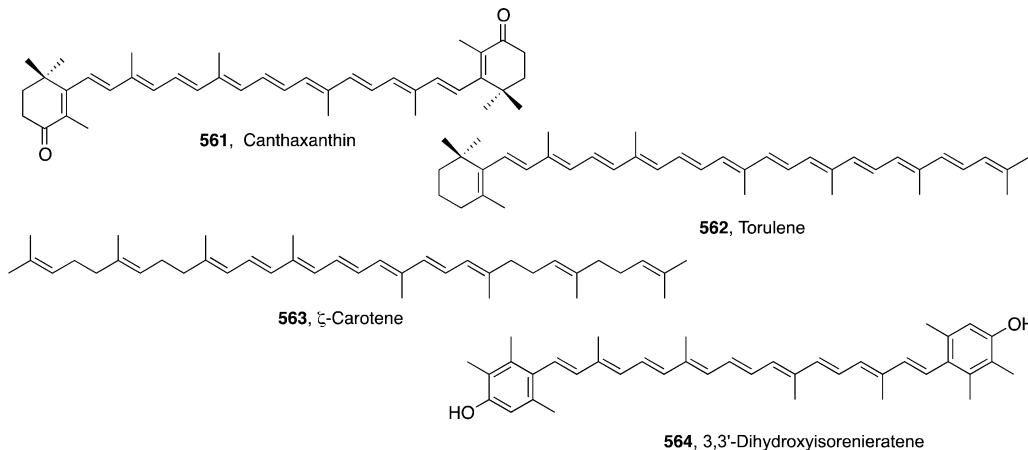


Figure 32. Carotenoids with well-characterized antioxidant properties.

in the complex mixture.⁵²⁹ Unsaturated structures containing a nitroxide radical have also been detected by EPR from the treatment of β,β -carotene (4) with nitrite anion.⁵³⁰ Peroxynitrite (produced from superoxide and nitric oxide radical) causes nitration of the aromatic ring protein tyrosine residues and damages low-density lipoprotein (LDL), lipids, and DNA. Reaction of peroxinitrite with capsanthin (555), isolated from paprika, and with fucoxanthin (497), a major carotenoid from brown algae, produced a series of nitroderivatives and double-bond isomers at various positions of the polyene chain (556–560, Figure 31).⁵³¹ Moreover, because these two carotenoids (498 and 555) inhibited the nitration of tyrosine by peroxinitrite,⁵³¹ they may have the potential to reduce the risk of cancer induced by reactive nitrogen species. Other nitration products of astaxanthin (545, Figure 29), including apo derivatives and geometric isomers, have been characterized from the mixture of products obtained after scavenging astaxanthin with peroxynitrite.⁵³²

10.4. Antioxidant Properties of Carotenoids

Carotenoids may affect oxidative processes in biological systems including food by radical scavenging through two mechanisms: (1) reduction as in the case of most ROS (hydroxyl radicals, alkoxy radicals, and peroxy lipid radicals and phenoxy radicals), which are scavenged by β,β -carotene, or (2) oxidation as in the case of the superoxide anion, which is efficiently scavenged by astaxanthin.⁵³³

10.4.1. Reduction Processes. Three reaction pathways have been proposed for the reaction of carotenoids with oxidizing radicals: (1) electron transfer to produce radical cations, (2) radical adduct formation, and (3) hydrogen atom transfer.

Carotenoid radical cations have lifetimes in the millisecond range and absorb at around 1000 nm in the near-infrared region. Radical adducts (absorption at $\lambda_{\max} \approx 520$ nm) are usually quenched by radical recombination, generating neutral products. Finally, hydrogen atom transfer to other radicals produces carotene radicals. The β,β -carotene radical structure was detected by time-resolved absorption spectroscopy by hydrogen atom abstraction of β,β -carotene (4) with hydroxyl radicals at 25 °C in a 4:1 CH₃CN/THF mixture. It was characterized as a short-lived transient species (150 ns lifetime under anaerobic conditions) with absorption maximum at $\lambda_{\max} \approx 750$ nm.⁵³⁴ While radical adducts are the most important products of radical scavenging, carotenoid radical species might

be generated under conditions of extreme oxidative stress.⁵³³ Additional radical species formed from β,β -carotene (4) have been characterized: radical anion pair, $\lambda_{\max} \approx 750$ nm, several hundred microsecond lifetimes; and radical anion, $\lambda_{\max} \approx 880$ nm, a few tens of microsecond lifetimes.⁵³⁴

The balance between electron donation and electron acceptance (ionization energy and electron affinity) to form radical cations or radical anions, respectively, varies among the carotenoids. The longer lived radical cations engage in one-electron reactions with a range of antioxidants (polyphenols, chromanols), including other carotenoids. Pulse radiolysis studies have established the relative ease of electron transfer for seven biologically relevant carotenoid radical cations: astaxanthin (545, Figure 29) > 8'-apo- β -carotenal (13, Scheme 2) > canthaxanthin (561, Figure 32) > lutein (491, Scheme 60) > zeaxanthin (492, Scheme 60) > β,β -carotene (4, Scheme 60) > lycopene (481, Scheme 60). Lycopene (481) is the strongest reducing agent (the most easily oxidized), and astaxanthin (545) is the weakest and acts as electron acceptor. The radical cations of the visual carotenoids, lutein (491), and zeaxanthin (492) are reduced by lycopene (481) but not by β,β -carotene (4).⁵³⁵ By monitoring the reaction of carotenoid radical anion with another carotenoid in hexane and benzene, an ordering of the reduction potential, and thus an antioxidant hierarchy of a variety of carotenoids has been established. The increase in conjugation and, for xanthophylls in benzene, the presence of carbonyl groups correlates with increasing reduction potential.⁵³⁶ Computational studies of a series of 19 carotenoids in polar and nonpolar solvents confirmed the experimental trends in oxidation potential, and predicted for torulene (562, Figure 32) the highest electron-donating capability, followed by lycopene (481).⁵³⁷ The reduction potentials of carotenoid radical cations in different solvents have been compared using their reactivity with retinol radical cation, a species generated by laser flash photolysis. The shorter polyene retinol radical cation has the lowest and β,β -carotene (4) the highest reduction potential, whereas zeaxanthin (492), astaxanthin (545), and 8'-apo- β -carotenal (13) have intermediate values in this experiment.⁵³⁸

Femtosecond transient absorption spectroscopy and quantum chemical computations of the excited states of the radical cations of β,β -carotene (4) and lutein (491) have characterized low-lying states (D₂ or D₃) below the near-IR absorbing excited state (with a strong D₀ to D₂ absorption band at $\lambda_{\max} \approx 800$ –1000 nm). In analogy to the neutral carotenoids, the excited-

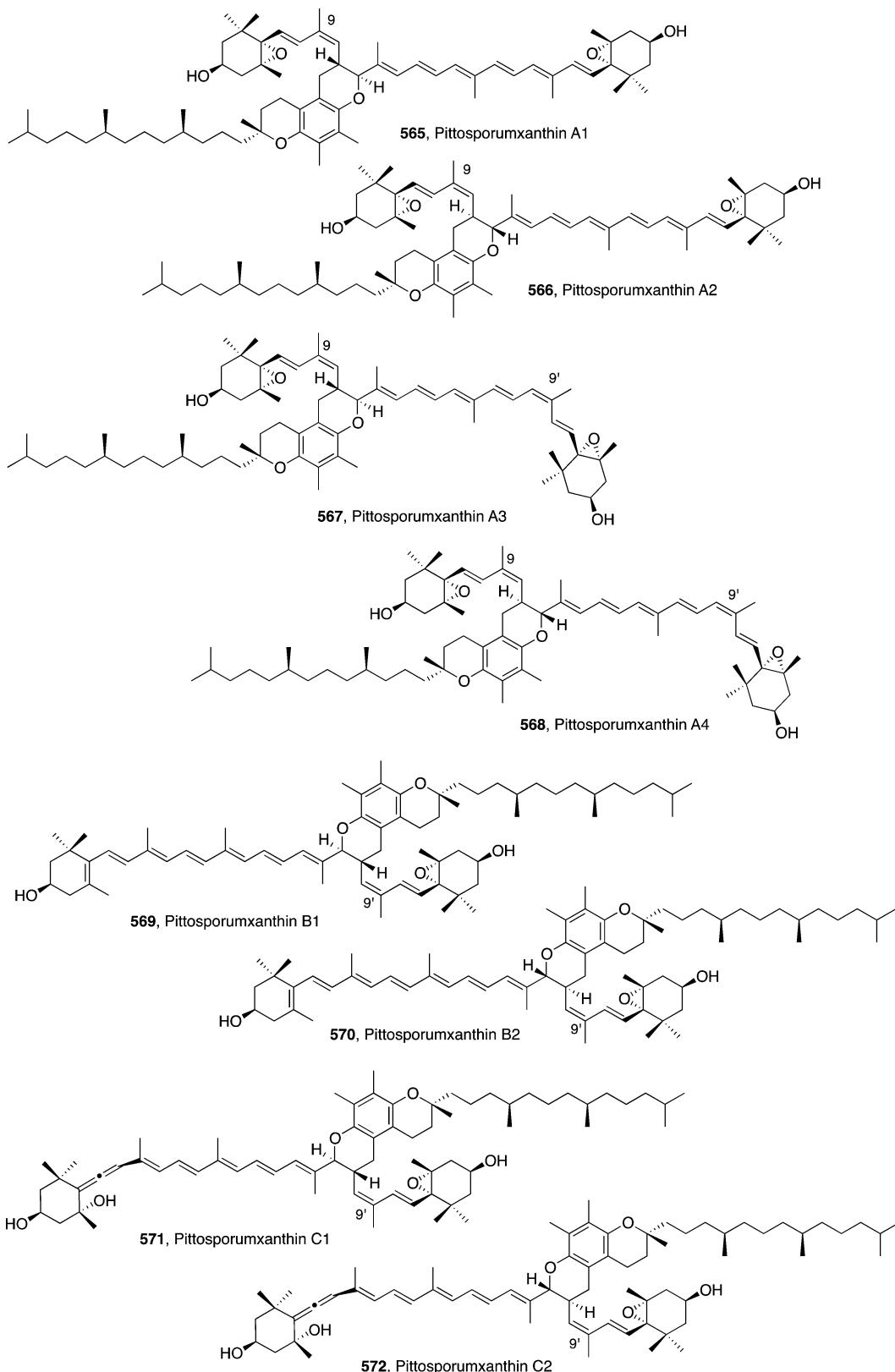


Figure 33. Xanthophyll–tocopherol adducts isolated from *Pittosporum tobira*.

state lifetimes of the radical cations (in the order of milliseconds) decrease as the number of conjugated double bonds increases.⁵³⁹

Radical formation by proton loss from radical cations (proposed to occur also in PSII samples after illumination at

20 K)⁵⁴⁰ has been theoretically examined as a function of length of the conjugated chromophore (from $N = 9$ to $N = 15$ double bonds).⁵⁴¹ A preference for the abstraction of the allylic methylene protons at the end of the conjugated chain was predicted for most of the symmetrical carotenoids (lycopene

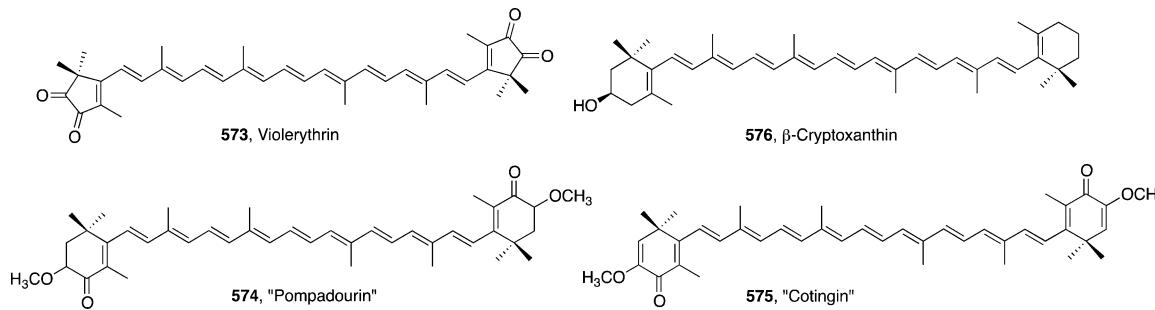


Figure 34. Selected carotenoids responsible for the coloration of animals.

481, spheroidene 507, spheroidenone, 509; see Figure 22). For a maximum conjugation of $N = 15$ (bis-dehydrolycopenes), the proton loss could occur from any of the 10 methyl groups, preferably the group at C1 or C1'.⁵⁴¹

Laser flash photolysis studies of the reactions of a range of carotenoids (β,β -carotene 4, ξ -carotene 563, Figure 32, lycopene 481, and synthetic analogues with shorter or longer polyene chains up to decapreno- β -carotene) with acylperoxy radicals, derived from the α -cleavage of carbonyl compounds, in polar and nonpolar solvents, revealed that carotenoid addition radicals do not react with oxygen, and thus the observed pro-oxidant effects of carotenoids must be attributed to some other mechanism.⁵⁴²

3,3'-Dihydroxyisorenieratene (564, Figure 32) has recently been characterized as superior antioxidant and photoprotective agent.⁵⁴³ In fibroblasts 564 prevented photooxidative damage, inhibited UV-induced lipid oxidation, suppressed heme oxygenase-1 (HO-1) expression, and prevented the formation of thymidine dimers.⁵⁴³ Its exceptional antioxidant activity was ascribed to its properties as a radical scavenger, which might result from the formation of an intermediate quinone structure with extended conjugation. This carotenoid was isolated for the first time from the bacterium *Streptomyces mediolani* but is also present in the membrane of *Brevibacterium linens*, a species used in the production of certain cheeses.

10.4.2. Oxidation Processes. The most important oxidative scavenging of carotenoids is the reduction of superoxide radical anion to generate the radical anion of the carotenoid and molecular oxygen. DFT studies support that carotenoids can oxidize superoxide radical anion in apolar media by acting as electron acceptors in reactions that are controlled by diffusion, and prevent the formation of oxidant ROS.⁵⁴⁴ In this study, astaxanthin (545, Figure 29) was a better quencher than lycopene (481).

10.4.3. Antioxidation Effects in Nature. In nature, carotenoids antioxidant effects are dependent upon kinetic or thermodynamic networks established with α -tocopherol, ascorbate, plant polyphenols, and other carotenoids.⁵³³ Synergistic effects of xanthophylls and carotenoids as antioxidants in membranes are possible if the hydrophilic carotenoids anchor in the water/lipid interface to facilitate electron transfer. It has been found that astaxanthin molecules (545) anchored to membranes in phosphatidylcholine liposomes may function as "molecular wires" scavenging radicals in the interface and facilitating electron transfer to nonpolar and more reducing carotenoids in the membrane interior.⁵⁴⁵

In the one-electron kinetically controlled antioxidant networks, carotenoids scavenge radicals.⁵³³ Structures suggestive of α -tocopherol radical quenching by epoxy carotenoids have been characterized from the seeds of the tree *Pittosporum tobira*, a

species rich in these xanthophylls. The structure correspond to cycloaddition products of several epoxy carotenoids with α -tocopherol [from (9Z)-violaxanthin, compounds 565 and 566; from (9Z,9'Z)-violaxanthin, compounds 567 and 568; from (9Z)-antheraxanthin, compounds 569 and 570; and from (9'Z)-neoanthin, compounds 571 and 572].⁵⁴⁶ These C69 carotenoids, named pittosporumxanthins A, B, and C (Figure 33), are present in nature as diastereomeric compounds at the fusion carbons. The absolute configuration of the separated diastereomers was established by CD.

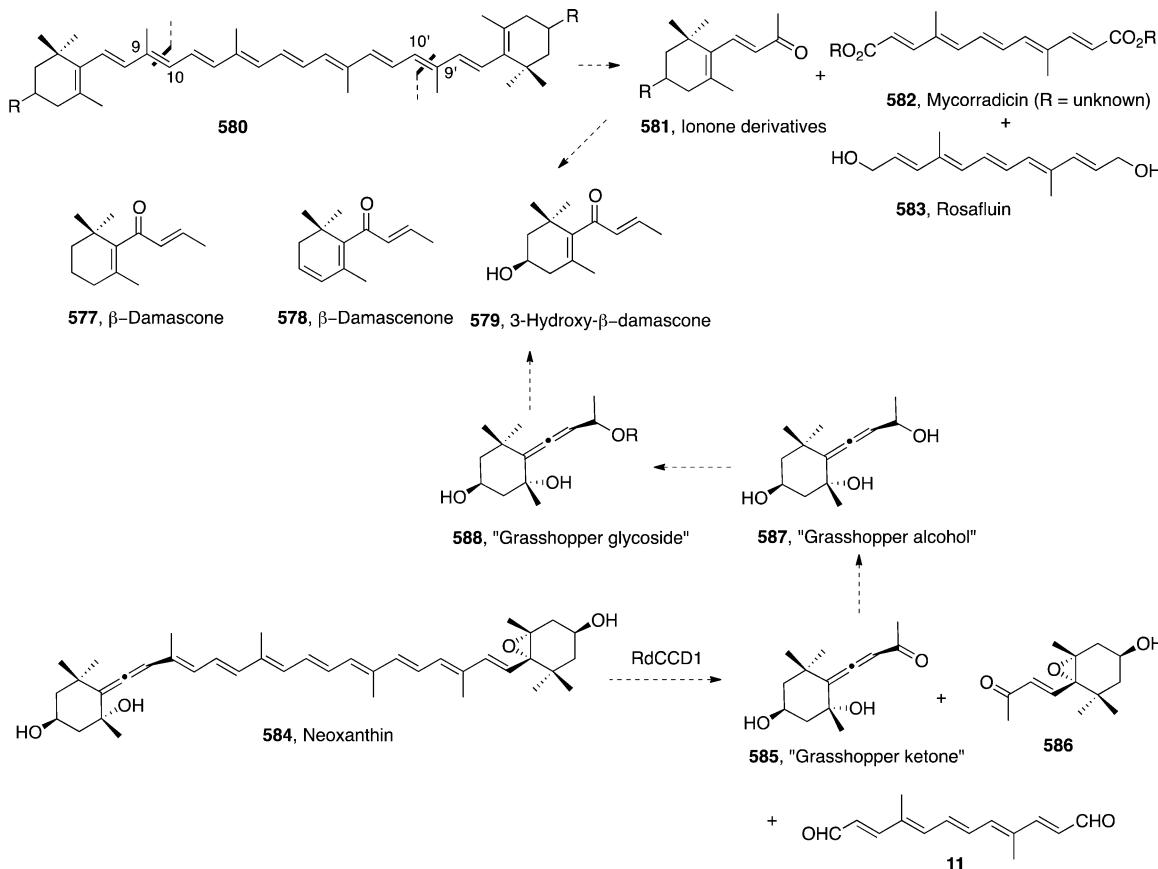
In the thermodynamically controlled antioxidant networks, carotenoids serve as color indicators of good antioxidant status, as might be the case for birds' plumage colors (vide infra).⁵³³

10.5. Color, Animal Behavior, Odor, Reproduction, and Survival

10.5.1. Color. Carotenoids produce the yellow colors of the pigments found in fruits, vegetables,⁵⁴⁷ and flowers.⁵⁴⁸ The pigmentation depends not only on the accumulation of carotenoids, but also on the transcriptional regulation of genes involved in carotenoid synthesis and degradation and on the control of carotenoid storage.⁵⁴⁹ Interestingly, the structures of the carotenoids found in different varieties of roses⁵⁴⁸ reflect the breeding partners used, with modern yellow roses showing greater proportion of xanthophylls.

Bird feathers, tropical fish, and crustaceans are also endowed with vivid colors based on carotenoids (Figure 34). Most carotenoids in organic solvents display yellow to red colors, and a few of them are blue. The blue carotenoid violerythrin (573, Figure 34) derives its color from a combination of the effects of a C_2 -symmetric undecaene skeleton conjugated to carbonyls in the cyclic end group, an almost coplanar *s-trans* conformation, and the presence of the cross-conjugated carbonyls. The solution and crystal structures adopted by the carotenoid were confirmed by ultrafast time-resolved absorption spectroscopy.⁵⁵⁰ The burgundy color plumage of the male *Pompadour cotinga* is ascribed to the presence of rare ring-substituted methoxy carotenoids (such as "pompadourin" 574 and "cotingin" 575, Figure 34) together with canthaxanthin (561, Figure 32) and astaxanthin (545, Figure 25). The absolute configuration of the chiral centers in the case of 574 remains unknown.⁵⁵¹

The coloration of flowers and animals by this group of yellow to purple dyes increases their attractiveness for pollination, seed dispersal, and sexual attraction.⁵⁵² About 30 different carotenoids have been identified in birds' plumage, and their concentration has been shown to decrease during migratory periods.⁵⁵³ Carotenoids in birds' plumage, as reflected by their color and color intensity, seem to be indicators of good antioxidant status and health.⁵³³ The sexual ornaments in the

Scheme 61. Apocarotenoids Related to Rose Odor and Possible Biogenesis of β -Damascenone (578) from Neoxanthin (584)

breast plumage of great tits, which are due to carotenoids, may reliably signal sperm quality through the males' capacity to protect their sperm from oxidative stress.⁵⁵⁴ The color of the bill of male blackbirds, which is due to the presence of a mixture of carotenoids, among them α -carotene (489, Scheme 60), β,β -carotene (4, Scheme 60), β -cryptoxanthin (576, Figure 34), zeaxanthin (492, Scheme 60), and lutein (491, Scheme 60), is considered a sexually selected trait. Experimental activation of the immune system resulted in the reduction of the expression of the carotenoid content in these animals, which indicates that a trade-off exists between sexual advertisement and immune activation.⁵⁵² Studies in zebra finches agree with the assumption that immune function can be limited by carotenoid availability in a species with carotenoid-dependent ornamentation and that females prefer the most carotenoid-rich males.⁵⁵⁵ A study of carotenoid content and accumulation in tissue of zebra finches confirmed differences in composition and concentration of the pigments in males and females, which might indicate different life-history strategies for their use.⁵⁵⁶ In fact, discrimination among the colorful males was linked to female plasma carotenoid levels.⁵⁵⁷

The facial skin yellow color of the rare Egyptian vulture *Noephron percnopterus* is due to the accumulation of carotenoids obtained from faeces of ungulates, which are transported in the plasma.⁵⁵⁸ HPLC analysis revealed that lutein (491) accounts for over 95% of the total carotenoid content of the skin (with small quantities of zeaxanthin 492, Scheme 60).

10.5.2. Odor. C₁₃-apocarotenoids such as β -ionone (10), β -damascone (577), and β -damascenone (578, Scheme 61) are odoriferous substances present in very small amounts (less than

1%) that contribute to more than 90% of the odor content of rose oil.⁵⁵⁹ The Damask rose (*Rosa damascena* Mill) and the Cabbage Rose or Rose de Mai (*Rosa centifolia* L.) are the two main species cultivated for the production of rose oils and absolutes.⁵⁵⁹ These C₁₃-apocarotenoids are biogenetically derived from carotenoids by oxidative degradation processes mediated by CCEs (see section 3). Double cleavage of the C9=C10 bonds of carotenoids and xanthophylls (general structure 580, Scheme 61) generates a C₁₄ dialdehyde (rosafluene, 11 or its diol rosafluin, 583) and oxidized derivatives (such as mycoradicin, 582).⁵⁶⁰ The accompanying C₁₃ fragments (ionones) and their derivatives, such as the components of the rose oil, contribute to aroma and scent of flowers, fruits, and vegetables. Carotenoid cleavage oxygenases from marine proteobacteria were shown to cleave monocyclic carotenoids and even acyclic (lycopene) and bicyclic C₄₀ and C₅₀ carotenoids (such as decaprenoxanthin, 503, Figure 21).⁵⁶¹

A member of the CCE genes, *RdCCD1*, has been isolated from *R. damascena* and expressed in *E. coli*.⁵⁶² In vitro studies have revealed that *RdCCD1* is a rather promiscuous enzyme that cleaves a variety of carotenoids at the C9=C10 and C9'=C10' positions to generate the C₁₄ dialdehyde rosafluene (11) and two C₁₃ products (585 and 586, Scheme 61). Both β -ionone (10) and "grasshopper ketone" (585) were detected from the incubation of *RdCCD1* with β,β -carotene (4) and neoxanthin (584), respectively. The reduction of "grasshopper ketone" (585) to the corresponding alcohol 587 and glycosylation of the latter would generate 588, the precursor of β -damascenone (578), possibly via the odorless 3-hydroxy- β -damascone intermediate (579), which is present in higher amounts in the plant.⁵⁶²

Scheme 62. Apocarotenoids of Commercial Value Obtained from Plants

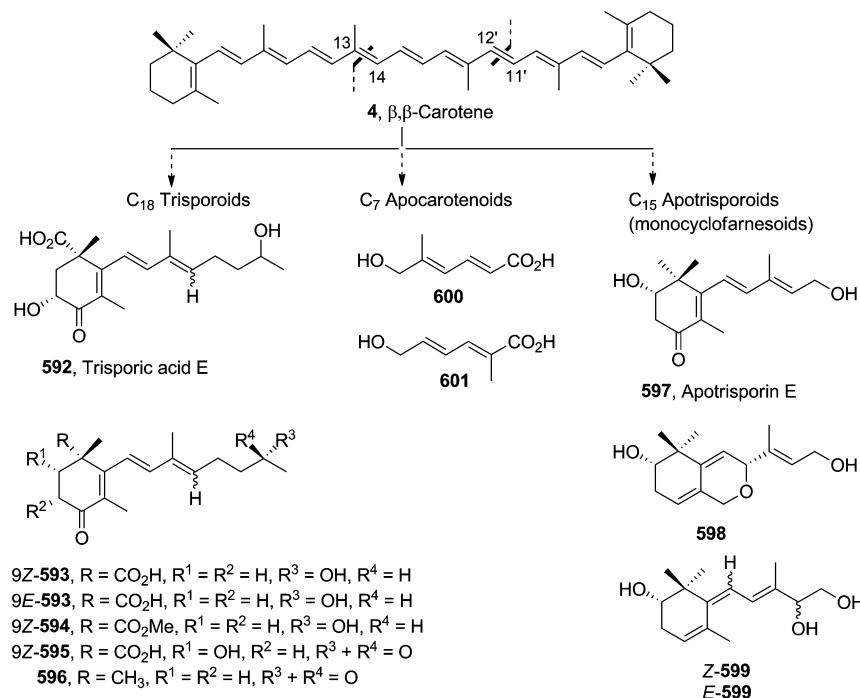
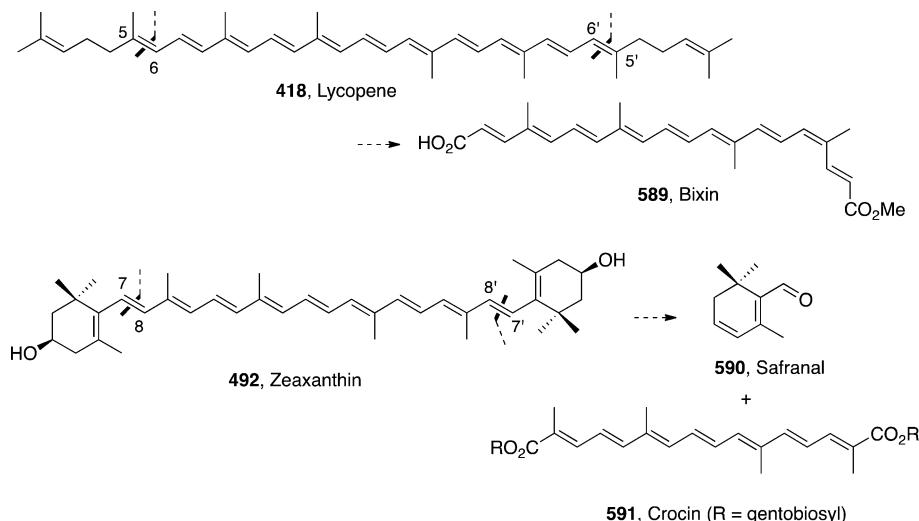


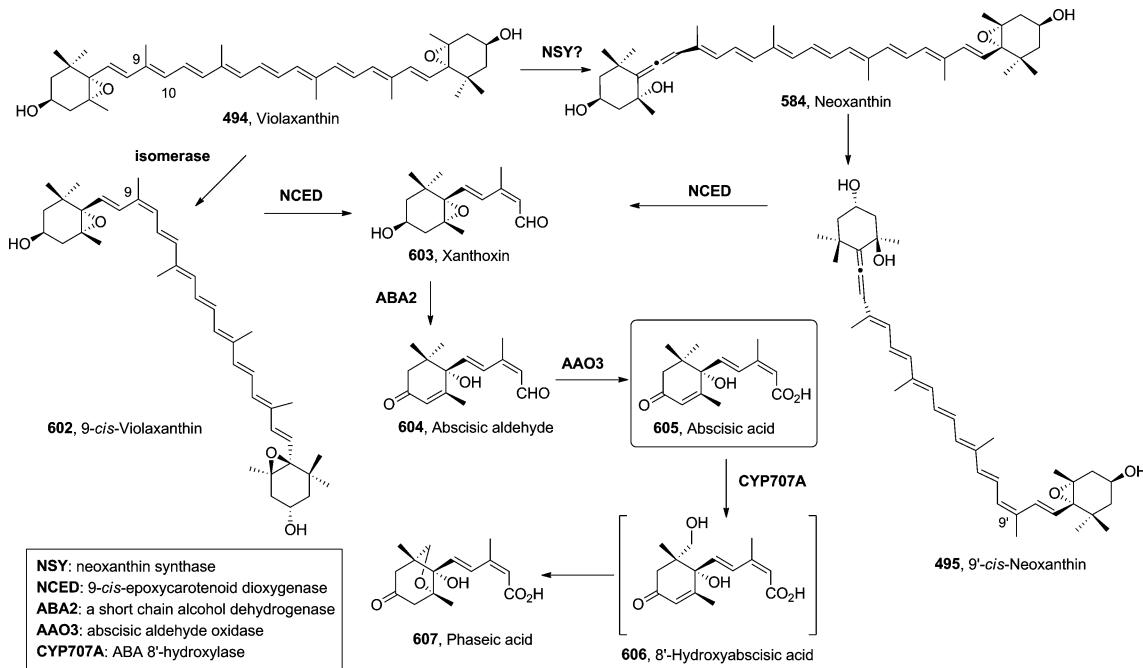
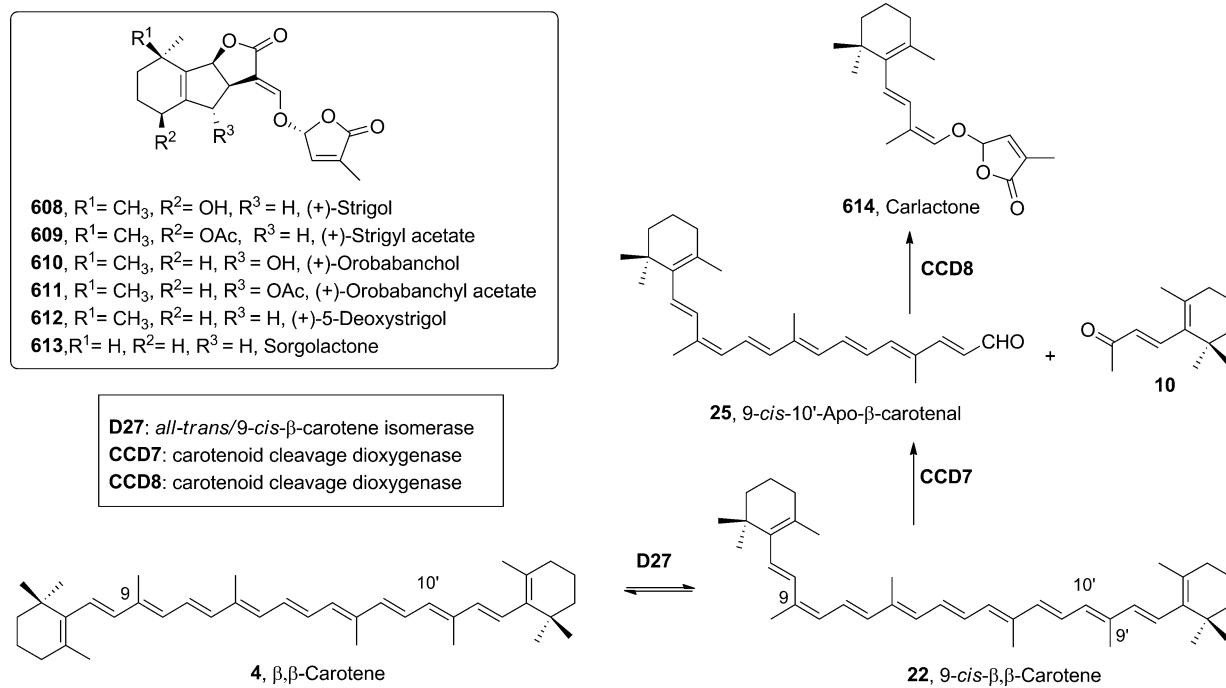
Figure 35. Apocarotenoid pheromones from Mucorales.

Two apocarotenoid pigments of commercial value are biosynthesized by plant reproductive tissues. Bixin (589, Scheme 62), the monomethylester of the C_{24} dicarboxylic acid norbixin, derives from the central part of C_{40} lycopene (418) by enzymatic cleavage of the $C5=C6$ and $C5'=C6'$ bonds. It is obtained from the seeds of the bush *Bixa orellana* and is used as a food colorant and lipstick ingredient, covering a range of yellow to red colors. Saffron, contained in the dry stigmas of *Crocus sativus*, is a C_{20} polyene derivative (crocin 591, the digentibiosyl ester of dicarboxylic acid crocetin), which originates from C_{40} zeaxanthin (492) via cleavage of the $C7=C8$ and $C7'=C8'$ bonds, that also produces C_{10} safranal (590) and is employed for coloring and seasoning food, with an estimated cost of \$1000–2000 kg⁻¹.^{560,563}

10.5.3. Sexual Pheromones. C_{13} apocarotenoids (α -ionone and derivatives, 581, Scheme 61) act as sexual

attractants for males of the Solanaceous fruit fly *Bactrocera latifrons* when combined with phenolic volatiles.⁵⁶⁴

Double cleavage at the $C13=C14$ and $C11'=C12'$ of β,β -carotene (4) provides a series of apocarotenoids that are considered as pheromones in the sexual interaction of fungi of the order Mucorales.⁵⁶⁵ These fungi belong to either the (+) or the (-) sex and are not distinguishable by their morphology but exchange sex-specific diffusible signals when mycelia of opposite sex grow near each other ("mated cultures"). The exchange of signals increases the β,β -carotene content and triggers the morphological program of the sexual cycle. In fact, trisporic acid C isolated from mixed cultures of *Blakesea trispora* of different sex was the first sexual pheromone characterized,⁵⁶⁶ and several congeners have recently been reported from other Mucorales.⁵⁶⁷ The structures of selected apocarotenoids isolated from culture media of *Blakesea trispora*

Scheme 63. ABA Biosynthesis and Catabolism to Phaseic Acid (607) in *A. thaliana*Scheme 64. Biosynthesis of Carlactone (614) in *Arabidopsis* and Structures of Strigolactones (608–613)

and *Phycomyces blakesleeanus* are shown in Figure 35.⁵⁶⁷ The cyclic compounds belong structurally to either the C₁₈ trisporoids (593–596, Figure 35) or the C₁₅ monocyclofarneoids groups (597–599). The acyclic C₇ methylhexadiene fragments 600 and 601 complete the degraded C₄₀ β,β -carotene skeleton precursor. The three classes of compounds are isolated in almost equimolar amounts from wild-type fungi cultures. Further support for the metabolic origin of these compounds by oxidation at the C13=C14 and C11'=C12' bonds of β,β -carotene (4) was obtained from studies on mutants devoid of β,β -carotene⁵⁶⁸ and from labeling studies.⁵⁶⁶

10.6. Phytohormones and Signaling Molecules

Important signaling molecules in plants also derive from the cleavage of carotenoids.^{560,569} 13-Apo- β -carotenone (20) affects the growth of root hairs in plants. Mycorradicin (582, Scheme 61) accumulates primarily in roots colonized by arbuscular mycorrhizal fungi (AMF).⁵⁶⁰ Abscisic acid (ABA, 605, Scheme 63) and strigolactones (Scheme 64)⁵⁷⁰ are the best characterized of the apocarotenoid phytohormones that influence processes as diverse as morphogenesis, seed dormancy, and environmental adaptation of plants.⁵⁷¹ The host root-derived signaling molecules like strigolactones, which

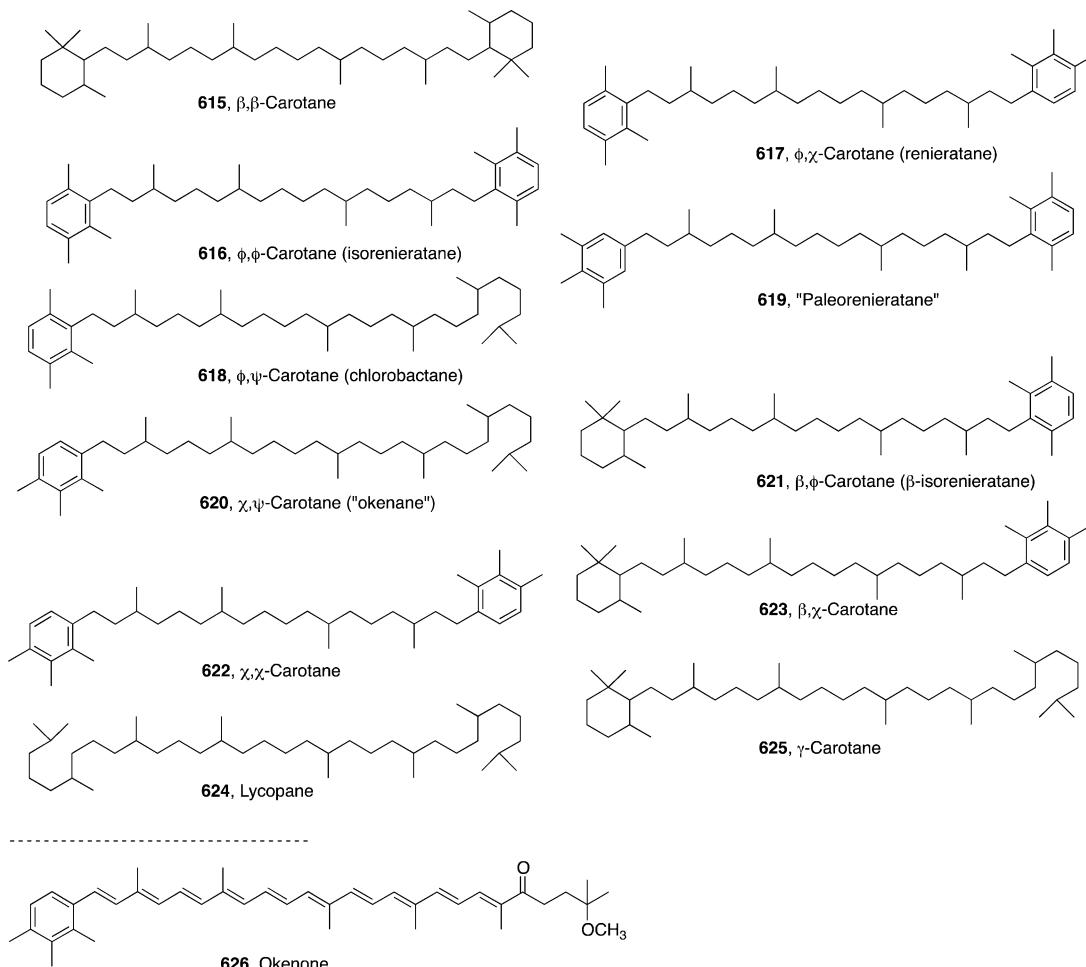


Figure 36. Saturated biomarker carotenoids isolated from marine sediments, and structure of okenone (626).

trigger AMF hyphal branching, are of major importance for the chemical communication between plants and AMF and supports their mutualistic symbiotic associations with the roots of more than 80% of land plants.^{572,570} This chemical communication is not only of interest for basic studies in plant physiology and plant–microbe interactions, but also for commercial applications.

ABA (605) is a C₁₅ apocarotenoid derived from the cleavage of xanthophylls.⁵⁷³ ABA (605) has been linked to several physiological processes in plants, among them the regulation of seed dormancy and germination, and the response to abiotic stress (temperature, light, drought).⁵⁷⁴ Its biosynthesis from 9'-*cis*-neoxanthin (495) and 9'-*cis*-violaxanthin (602) through xanthoxin (603) and abscisic aldehyde (604) by the action of the enzyme 9'-*cis*-epoxycarotenoid dioxygenase (NCED) has been elucidated in *A. thaliana*.⁵⁷³ Phaseic acid (607) is formed by catabolism of ABA via 8'-hydroxyabscisic acid (606), and the control of its formation might contribute to regulate the concentration of the phytohormone.

Strigolactones (608–613, Scheme 64) such as deoxystrigol are phytohormones released by plant roots that trigger seed germination of root parasitic weeds (the genera *Striga* and *Orobanche* are considered the most damaging agricultural agents in the developing world) and can induce hyphal branching in AMF before symbiosis.^{570,571} Recombinant *Arabidopsis* carotenoid cleavage dioxygenase protein 7 (CCD7) exhibits a specific C9'=C10' cleavage activity in

vitro converting β,β-carotene (4) to 10'-apo-β-carotenal (9) and β-ionone (10).⁵⁷⁵ C₁₈ 13-apo-β-carotenone (20, Scheme 2), presumably formed from 10'-apo-β-carotenal (9), was at first considered the biosynthetic precursor of the strigolactones.⁵⁷⁶ However, in *Arabidopsis*, the biosynthesis of carlactone (614, Scheme 64), a plant hormone related to the strigolactones, requires the isomerization of β,β-carotene (4) to 9'-*cis*-β,β-carotene (22) by the action of carotene isomerase D27 (DWARF27), an iron containing protein. The enzymes responsible for formation of the carbon skeleton of carlactone (614) from β,β-carotene (4) are also members of the CCD family, nonheme iron dioxygenase enzymes. CCD7 cleaves 9'-*cis*-β,β-carotene (22) to 9'-*cis*-10'-apo-β-carotenal (25, Scheme 3), whereas CCD8 produces carlactone (614).⁵⁷⁷ CCD8 also cleaves *trans*-10'-apo-β-carotenal (9) to 13-apo-β-carotenone (20), but at a slower rate.⁵⁷⁷

The biological activities of the apocarotenoids derived from β,β-carotene and other provitamin A carotenoids have been discussed in section 3.1. 10'-Apo-lycopenoic acid, presumably formed from 10'-apo-lycopenal (which was shown to be produced in ferrets through metabolism of lycopene by CCEs),^{52a} was found to transactivate RARs with relatively high potency and inhibits 4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK)-induced lung tumor growth in the A/J mouse model.⁵⁴ 10'-Apo-lycopenoic acid impacts adipose tissue biology via RARs,⁵⁷⁸ increases sirtuin 1 mRNA and protein levels, and decreases hepatic fat accumulation in *ob/ob*

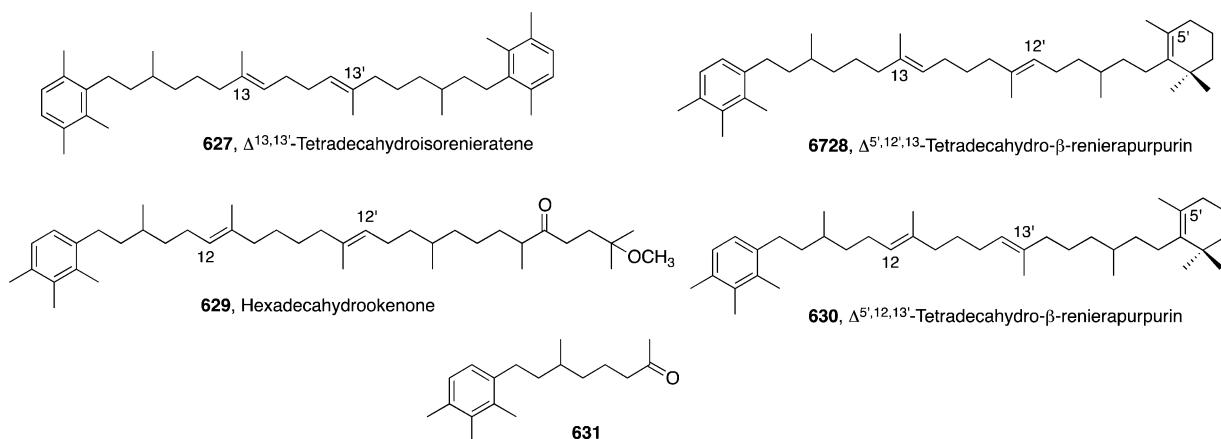


Figure 37. Partially saturated biomarker carotenoids isolated from marine sediments.

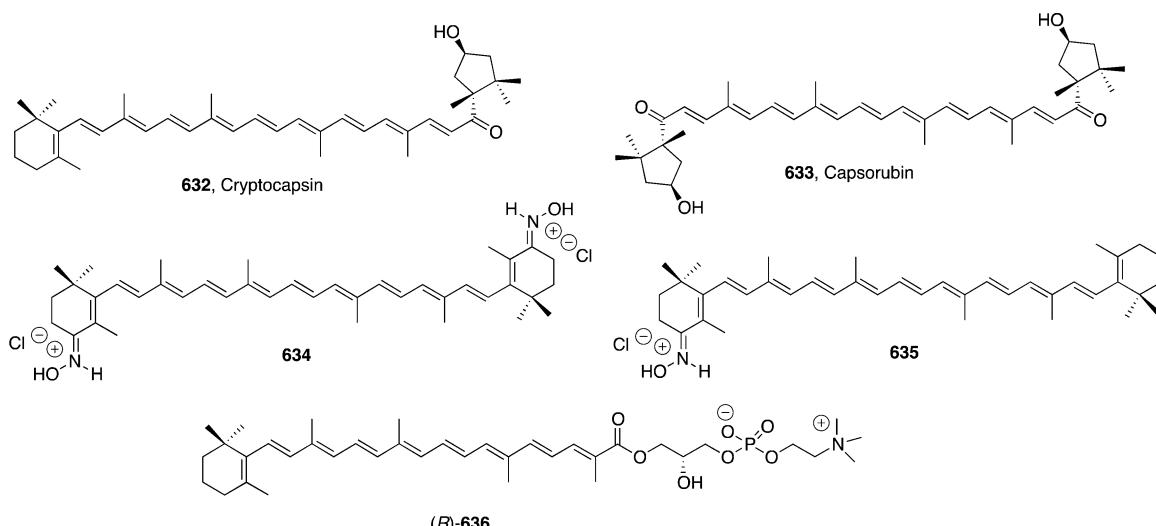


Figure 38. Some natural and synthetic carotenoids that form aggregates.

mice.⁵⁷⁹ Crocetin (the dicarboxylic acid of **591**, Scheme 62) induces cytotoxicity and enhances vincristine-induced cancer cell death via p53-dependent and p53-independent mechanisms.⁵⁸⁰ Bixin (**589**, Scheme 62) has been reported to activate PPARs.⁵⁸¹ Interestingly, ABA (**605**, Scheme 63) has been identified as an endogenous pro-inflammatory cytokine in human granulocytes. It stimulates several functional activities of human granulocytes through a pertussis toxin (PTC)-sensitive GPRC, protein kinase A activation, ADP-ribosyl cyclase phosphorylation, cyclic ADP-ribose overproduction, and increase in intracellular Ca^{2+} concentration.⁵⁸² The regulation of the nuclear localization of the COP1 ubiquitin ligase, which in part determines the levels of light regulators such as HYS, is a new activity of strigolactone discovered in a small-molecule screen.⁵⁸³

10.7. Carotenoids as Biomarkers

Specific carotenoids are preserved intact in ancient mineral sediments or crude oils deposited under strictly anaerobic conditions. These compounds were produced by green sulfur bacteria (*Chlorobiaceae*), which are strict anaerobes that require light and hydrogen sulfide in stratified water columns to carry out photosynthesis, and therefore are considered as biomarkers for these photic zones (euxinic) in depositional environments.

The dominant biomarkers are the typical breakdown products of aromatic carotenoids, the latter also detected in the thermally least altered bitumens.⁵⁸⁴ Some reduced carotenoids with 2,3,6- and 2,3,4-trimethyl arylisoprenoid skeletons (among others, isorenieratane **616**, renieratane **617**, chlorobactane **618**, and okenane **620**, Figure 36) have been characterized in anoxic sediments.⁵⁸⁵ The C₄₀ carotenoids β -carotane (**615**), lycopane (**624**), and the tentatively identified γ -carotane (**625**) were also found as part of the aromatic hydrocarbon fractions of a stratified Palaeoproterozoic sea (1.64 GYr-old basin).⁵⁸⁴ The presence of the new carotenoid biomarker okenane (**620**), a partially reduced derivative of okenone (**626**), which is biosynthesized by *Chromatiaceae*, indicated that these phototrophic purple sulfur bacteria were present in the geological record together with communities of green sulfur bacteria (*Chlorobiaceae*), which biosynthesized the parent isorenieratene (**504**, Figure 22), β -isorenieratene, and chlorobactene (**505**, Figure 22).^{584,586}

Additional carotene derivatives (**627–631**, Figure 37) have been detected in sediments of sulfide-rich, anoxic environments.⁵⁸⁶ Using several spectroscopic techniques, it was shown that the partially unsaturated derivatives with stereogenic centers were present as mixtures of products (including the diastereoisomers at the stereogenic centers), which should rule out enzyme-mediated reactions to explain their diagenetic

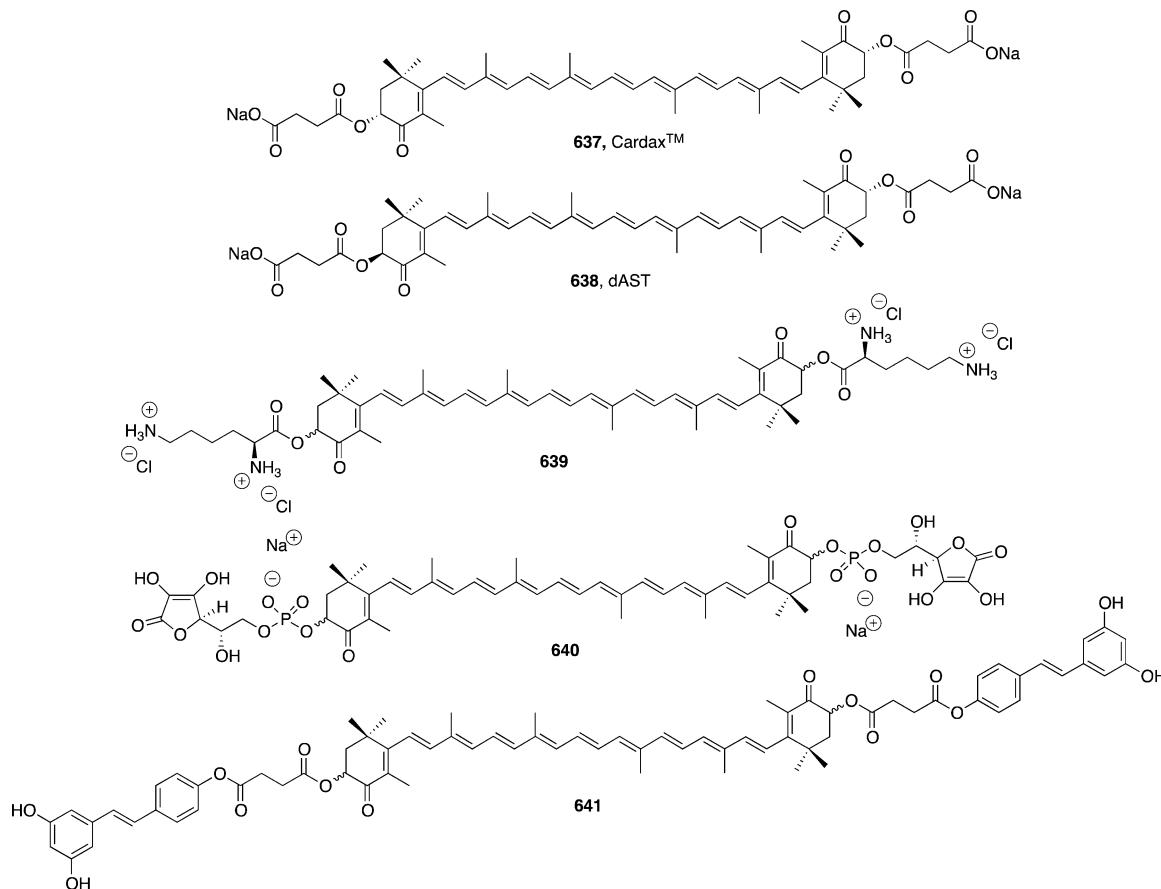


Figure 39. Synthetic hydrophilic carotenoid derivatives.

formation from the parent carotenoids. Instead, an indirect two-step mechanism (i.e., addition of H_2S followed by single-electron transfer reductive desulfurization of the allylic thiol by $\text{H}_2\text{S}/\text{SH}^-$ as electron donor) using sulfides produced by bacterial sulfate reduction was proposed to explain the saturation of several double bonds of the polyene chain. Thus, the transformation of labile, oxygen-sensitive carotenoids into their more stable reduced counterparts is considered a plausible mechanism for the preservation of sedimentary organic matter throughout geological history.⁵⁸⁶

11. CAROTENOID AGGREGATES

When dissolved in hydrated solvents, carotenoids aggregate due to their hydrophobic properties. Carotenoid aggregates are best viewed as associations of carotenoid surfactants in a water-based medium. The presence of hydrophilic groups on the carotenoid structure contributes to its surfactant properties.⁵⁸⁷ Two carotenoids that have been used as natural food colorants since ancient times are highly unsaturated diacid derivatives. Crocin (591, Scheme 62) is the only natural carotenoid that is soluble in H_2O . Bixin (589, Scheme 62) is water insoluble, and the potassium salt of its diacid derivative (norbixin) showed the typical surface and aggregation behavior of a bolaamphiphile.⁵⁸⁸

Using astaxanthin (545, Figure 29) as a model, two types of carotenoid aggregates were characterized: (1) *H* aggregates exhibit large hypsochromic shifts indicative of tight association with parallel orientation of the chromophores ("card-pack aggregates"); (2) *J* aggregates are characterized by bathochromic shifts due to the head-to-tail orientation of the interacting chromophores ("loose-type aggregates").⁵⁸⁹ The

relationship between the structure of the carotenoid and the observed optical properties is not yet clear,⁵⁹⁰ because most of the studies have focused on semisynthetic analogues (epimers, alcohol/ketones, acetates) of the κ -end group carotenoids capsanthin (555, Figure 31), cryptocapsin (632), and capsorubin (633, Figure 38). For example, capsorubin (633) forms *H*-type aggregates with small contributions of the *J*-type, whereas its double epimer called "epicapsorubin" exhibited a greater proportion of head-to-tail structures, which was interpreted as due to intramolecular hydrogen bonding. The alcohol derivative capsanthol, on the other hand, forms card-pak aggregates with a continuous chain of hydrogen bonds.⁵⁹¹

In addition to the dramatic changes in absorption spectra, these supramolecular assemblies exhibit induced Cotton effects of opposite sign (exciton couplet) relative to the isolated molecules ("supramolecular exciton chirality").⁵⁸⁹ Chiroptical spectra of carotenoid aggregates of configurationally chiral compounds show Cotton effects that are considerably higher than those of the individual molecules, which is due to the delocalization of excitation energy (exciton) over neighboring chromophores.

The biological relevance of carotenoid aggregates remains to be demonstrated, although they are present in lipid bilayers in various biological systems and might contribute to the physical and dynamic properties of lipid membranes. Their role in plant photoprotection and NPQ cannot be discarded, as the xanthophylls tend to assemble into aggregates.⁵⁹² The CD spectra of isolated chromoplasts are remarkably similar to those of the head-to-tail aggregates of acetylated xanthophylls in solution.⁵⁹³ Zeaxanthin forms dominant dimeric *J* structures

with some contribution from head-to-tail counterparts. Violaxanthin showed a mixture of structures with predominant H-type dimers. Lutein aggregates forming card-pack assemblies in which the molecules are tightly packed and oriented nearly parallel to each other.⁵⁹⁴ Astaxanthin forms octamers as aggregation units. In flower petals, J aggregates are almost exclusively formed, and they might contribute to recognition by insects. The presence of xanthophyll J aggregates allows plants to sense light in a wide spectral range.

Synthetic hydrophilic carotenoids have been made following two strategies: addition of a hydrophilic group to a carotenoid scaffold, and combination of a carotenoid and a hydrophilic compound.⁵⁸⁷ Examples of the first case are the oxime hydrochlorides of canthaxanthin (**634**) and echinenone (**635**, Figure 38), which prefer to arrange into J aggregates,⁵⁹⁵ and the lysophosphocholine adduct of an 8'-apo- β -carotenoic acid and choline (**636**)⁵⁹⁶ (the R enantiomer is shown in Figure 38).⁵⁹⁷ The latter might form an octameric helical P-screwed oligomer⁵⁹⁷ of optically inactive individual (R) units, according to its chiroptical properties.

The attachment of hydrophilic groups to the hydroxyl groups of xanthophylls, in particular astaxanthin (**545**, Figure 29), generated several hydrophilic derivatives. Bolaamphiphile Cardax, the disodium salt of astaxanthin disuccinate (**637**, Figure 39), which forms nonspherical aggregates, is commercially produced. Intervention studies to determine its antioxidant activities in aqueous formulations are ongoing. The same type of dicarboxylate derived from *meso*-zeaxanthin **638** has been incorporated into fatty acid-free human serum albumin, and the chiroptical signatures suggest the formation of mixed carotenoid–protein right-handed assemblies.⁵⁹⁸ The dilsine conjugate **639** surpasses crocin in water solubility, forming deep red solutions in water and aggregates at high concentrations. The ascorbic acid–astaxanthin conjugate with a phosphate linker **640** showed much higher antioxidant properties than the parent carotenoid. Other hydrophilic groups have been attached to the target carotenoid, including resveratrol in **641** (Figure 39), carbohydrates (maltose, mannitol, and sorbitol), citric acid, and glutathione.⁵⁸⁷

12. INTESTINAL ABSORPTION, TRANSPORT OF CAROTENOIDS, AND RECEPTORS

12.1. Intestinal Absorption and Transport

Nearly 60 carotenoids are consumed in the diet. In the mammalian digestive tract, carotenes and unesterified xanthophylls (nonspecific esterases catalyze xanthophyll ester cleavage in the gut) are solubilized into mixed lipid micelles in the lumen, absorbed by intestinal mucosal cells, and incorporated into chylomicrometers.⁵⁹⁹ Carotenoids and their metabolites are then secreted with transport chylomicrometers into the circulation and targeted to a variety of tissues, primarily eye macula, liver, lung, adipose, brain, prostate, and skin.⁵⁹⁹ More than 30 of the ingested carotenoids are found in blood, including some metabolites, although only lutein (**491**) and zeaxanthin (**492**, Scheme 60) are taken up from the blood into eye tissues. The selectivity in the uptake of carotenoids by tissues depends on the formation of complexes with carotenoid-binding proteins (CBPs). These proteins could operate at several levels: as cell surface receptors or transmembrane transport proteins, as metabolic enzymes, or as intracellular mediators. In some cases, they could simply contribute to protection and stabilization of the ligand.⁶⁰⁰

12.2. Invertebrate CBPs

The yellow color of silkworm *Bombyx mori* cocoons is due to the accumulation of lutein (**491**). After feeding on mulberry leaves, silkworm larvae absorb the carotenoids into the intestinal mucosa and transfer them to a hemolymphal lipoprotein called lipophorin. Lutein (**491**) is then transported to the silk glands by a lutein-specific CBP.⁶⁰¹ Intracellular CBP, the product of the Y (yellow blood) gene,⁶⁰² is a 33 kDa protein that in addition to lutein (88%) also binds β,β -carotene (9%) and α -carotene (3%) in the same proportion as the carotenoid composition of lipophorin. The absorption maximum of this ca. 1:1 carotenoid–protein complex in the visible ($\lambda_{\text{max}} \approx 436, 461$, and 493 nm) is 22 nm red-shifted as compared to the absorption of lutein (**491**) in hexane.⁶⁰³ Silkworm strains with modulated silk coloration, which is of economic significance for the textile industry, have been developed by genetic engineering.⁶⁰²

α -Crustacyanin isolated from carapace of the lobster *Homarus gammarus* is another invertebrate CBP that binds astaxanthin (**545**, Figure 29). The absorption maximum of the complex ($\lambda_{\text{max}} \approx 632$ nm) shows a large bathochromic shift of 160 nm and results in the deep blue color of the lobster (perhaps as a camouflage strategy), which turns into red upon boiling due to denaturation and release of astaxanthin ($\lambda_{\text{max}} \approx 472$ nm in hexane).⁶⁰⁴ The red-shift of the complex is caused by the exciton coupling due to the proximity of the astaxanthin chromophores (70%) and to protein-induced conformational changes and polarization effects (30%), as concluded from a combination of solid-state NMR with ¹³C-enriched α -crustacyanin, resonance Raman spectroscopy, and time-dependent DFT studies.⁶⁰⁵

12.3. Vertebrate CBPs

Receptors for transport of carotenoids are rather unspecific, although *cis*-isomers of carotenoids are considered more bioavailable than *trans*-isomers due to their greater solubility in bile acid micelles. The *cis* carotenoids could also be incorporated into chylomicrometers in preference to the *trans* isomers. The more hydrophobic carotenoids are associated with the plasma binding proteins VLDL and LDL, the xanthophylls with HDL, and both are delivered to target tissues via their interaction with cell surface receptors. This association is mutually beneficial as carotenoids might protect lipoproteins from free radicals that convert polyunsaturated fatty acids into hydroperoxides. Albumin and β -lactoglobulin also bind carotenoids in the serum and milk, respectively, but appear to do so with low affinity. Fatty acid-free human serum albumin (HSA) may bind lutein (**491**) and *meso*-astaxanthin.⁵⁹⁸ Salmon muscle α -actinin was identified as the only myofibrillar protein that accumulates astaxanthin (**545**, Figure 29).⁶⁰⁶

The ocular CBP is the π isoform of human glutathione S-transferase (GSTP1),⁵¹⁶ and binds zeaxanthin (**492**, Scheme 60) and *meso*-zeaxanthin (**535**, Figure 28) with high affinity and specificity. A lutein-binding protein from the Japanese quail liver (qLBP) has been purified (absorption maximum at $\lambda_{\text{max}} \approx 537$ nm, the second largest bathochromic shift of CBP after α -crustacyanin), but not yet fully characterized.⁶⁰⁰ StARD3, a member of the steroidogenic acute regulatory domain family (StRD), is the receptor for lutein (**491**) in the primate macula.⁵¹⁷ Carotenoids of the macula also bind to the taxol binding site of the β -tubulin subunit of microtubules, where they might modulate the dynamic instability of microtubules in the primate macula.⁶⁰⁷ Liver is, together with adipose tissue, a

major storage organ for carotenoids in animals and for conversion to vitamin A (1, see section 3). A 67 kDa cellular β -carotene-binding protein (CCBP) from ferret liver (with absorption maxima at $\lambda_{\text{max}} \approx 460, 482$, and 516 nm, ca. 32 nm bathochromic shift) has been isolated.⁶⁰⁰

Artificial β -barrels of lipocalin-type toroidal amphiphilicity models have been prepared and used for encapsulation of carotenoids,⁶⁰⁸ as a first step for potential targeted delivery of carotenoids to cells.

13. CHEMOPREVENTION AND HEALTH-RELATED EFFECTS OF CAROTENOIDS

The chemopreventive actions and health benefits to humans of carotenoid consumption is an unsettled issue among nutritionists and physicians. Whether the antioxidant effects of carotenoids when used as phytonutrients are also beneficial to human health⁶⁰⁹ is also uncertain.⁶¹⁰ In 1998, an IARC panel concluded that for humans "There is evidence for lack of cancer-preventive activity of β,β -carotene when used as supplement at high doses. There is inadequate evidence with regard to cancer-preventive activity of β,β -carotene at the usual dietary levels. There is inadequate evidence with respect to the possible cancer preventive activity of other individual carotenoids." However, the same panel concluded that there is sufficient evidence that β,β -carotene and canthaxanthin have cancer-preventive activities in certain animal models for liver, colon, pancreas, skin, and buccal pouch carcinogenesis.¹¹

Epidemiological studies for the last 30 years suggest little or no association between the total intake of fruit and vegetables and the risk of common cancers, including colorectal, breast, and prostate cancer, at least in well-nourished populations.⁶¹¹ Recent studies found no association between a high consumption of vegetables and fruits and pancreatic cancer risk in a large cohort study of 120 852 men and women in The Netherlands.⁶¹² Weak or nonsignificant association between fruit and vegetable consumption and the occurrence of coronary heart disease was deduced from epidemiological studies.⁶¹³ However, the reduction of blood pressure, which is an important cardiovascular risk factor, has been associated with intake of fruits and vegetables in controlled nutritional prevention trials.⁶¹³

A systematic review and metanalysis of randomized controlled trials (RCTs) concluded that nutritional primary or secondary prevention of cancer through β,β -carotene supplementation should not be recommended.⁶¹⁴ The Cochrane Collaboration study, which reviewed all RCTs in the literature, concluded that the treatment with β,β -carotene, vitamin A, and vitamin E may increase mortality from 1% to 8%.⁶¹⁵ The α -tocopherol β,β -carotene retinol (ATBC)⁶¹⁶ and β,β -carotene retinol efficacy trial (CARET)⁶¹⁷ chemoprevention trials have shown that β,β -carotene, either alone or in combination with vitamin A or vitamin E, actually increases lung-cancer incidence and mortality in heavy smokers and asbestos workers. The ATBC study (29 133 participants) recorded 18% more lung cancers and 8% more overall deaths in smokers taking β,β -carotene. The CARET efficacy trial (18 314 participants who were at high risk for lung cancer because of a history of smoking or asbestos exposure) was interrupted in January 1996 because there were 28% more lung cancers and 17% more deaths in smokers and asbestos workers relative to the placebo group. Smokers' metabolism of carotenoids might play a special role in cancer promotion, perhaps by converting antioxidant molecules to prooxidant molecules (see section

10.4). Follow-up studies indicated that the adverse effects persisted after stopping the administration of the β,β -carotene supplements, but the results were not statistically significant.⁶¹⁸ The possible cocarcinogenic properties of β,β -carotene on latent cancers are consistent with animal studies, which have found that β,β -carotene in BALB/c 3T3 rat lung induces phase I carcinogen-bioactivating enzymes, including activators of polycyclic aromatic hydrocarbons (PAHs), and oxidative stress.⁶¹⁹ Long-term antioxidant supplementation (including β,β -carotene at 6 mg per day) had no effect on health-related quality of life as demonstrated in SU.VI.MAX, a randomized, double-blind, placebo-controlled, primary prevention trial in a total of 8112 French participants.⁶²⁰

In a large prospective study of 1 028 438 participants (33 380 incident invasive breast cancers), the intakes of α -carotene, β,β -carotene, and lutein/zeaxanthin were inversely associated with risk of ER-, but not ER+, breast cancer. However, the results need to be interpreted with caution because it is unclear whether the observed association is real or due to other constituents in the same food sources.⁶²¹

In a subsample of women in the Women's Health Initiative, baseline levels of antioxidant nutrients (retinol, α -carotene, β,β -carotene, β -cryptoxanthin (576, Figure 34), lutein + zeaxanthin, lycopene, α -tocopherol, and γ -tocopherol) were not associated with risk of colorectal or colon cancer; however, a relatively high serum level of β,β -carotene was inversely associated with risk of colon and colorectal cancer in postmenopausal women.⁶²² In another study of 198 Italian low-risk patients, the dietary intake of carotenoids, notably α -carotene and β,β -carotene, was inversely related to nasopharyngeal carcinoma risk.⁶²³

Lycopene, an efficient single oxygen quencher,⁶²⁴ or its metabolites⁶²⁵ have been proposed as chemoprotective agents against prostate cancer.⁶²⁶ However, intervention studies did not recommend supplementation to influence prostate cancer progression.⁶²⁷ Moreover, higher plasma lycopene concentrations were not associated with prostate cancer risk.⁶²⁸ Lycopene also plays an important role in the control of adipose tissue metabolism, inhibiting proinflammatory cytokine and chemokine expression and reducing the risk of developing cardiovascular disease in men. In a study in older men, no correlation was found between lycopene levels and a moderate increase in cardiovascular disease risk. Other carotenoids, as well as vitamin A, were also included in the study, and the risk of cardiovascular disease correlated with higher concentrations of plasma lutein/zeaxanthin (but not β -cryptoxanthin 576, α -carotene, and β,β -carotene) and retinol.⁶²⁹

A 1998 evaluation panel of IARC concluded that there is evidence suggesting lack of cancer-preventive activity of vitamin A in humans for cancers of the upper aerodigestive tract, lung, breast (postmenopausal women), colorectal, bladder, prostate, and stomach; yet the same panel found that there is limited evidence for cancer-preventive activity of retinyl esters in some experimental animal models.¹¹ Note that in all of these cases only monotherapy has been explored.

Further, no association was found between intake of vitamin A and carotenoids and melanoma risk in a large prospective cohort study (69 635 men and women participating in the vitamins and lifestyle, VITAL).⁶³⁰ Photoprotective effects of dietary components such as β,β -carotene or lycopene require several weeks after consumption of food rich in these pigments, due to the physiological turnover time of skin, and thus they are less efficient for photoprotection than topical sunscreens.⁶³¹

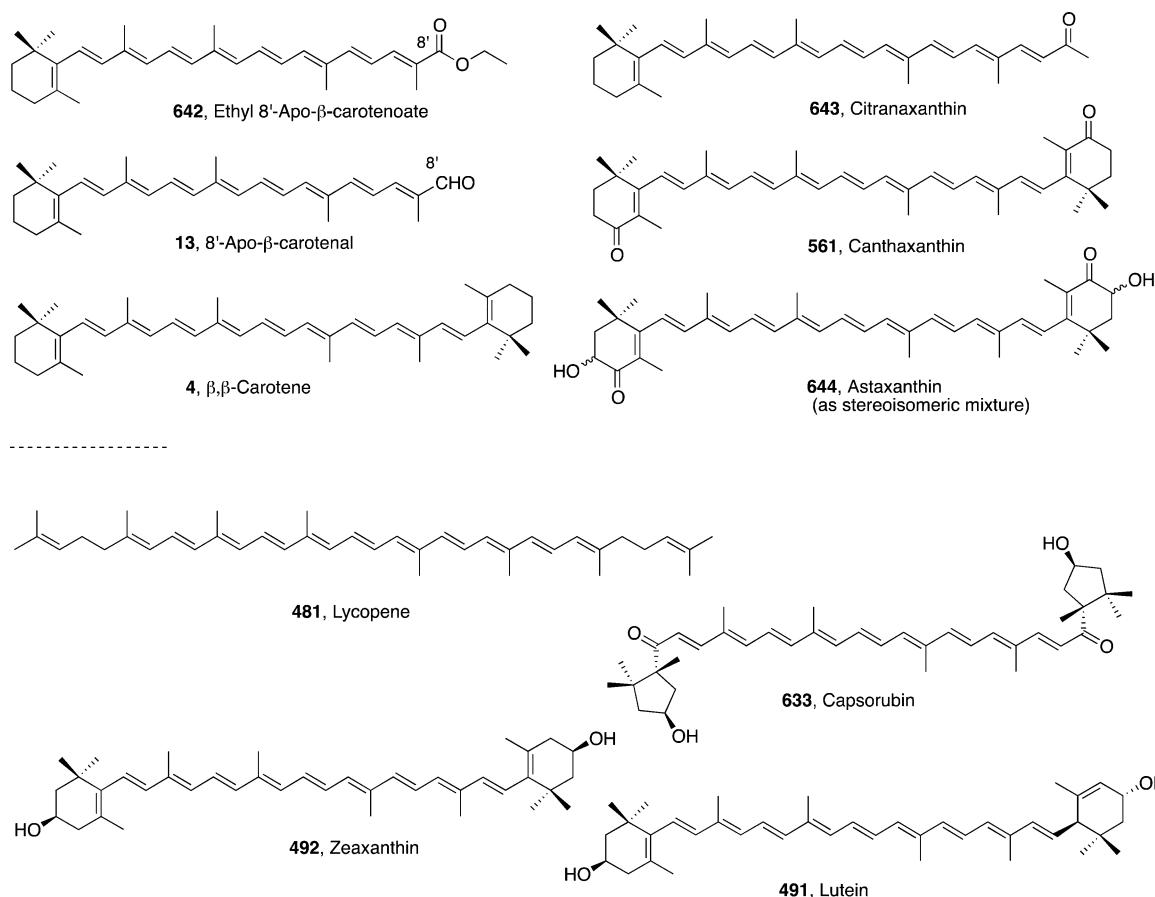


Figure 40. Carotenoids produced for commercial use in the nutraceutical industry.

Likewise, the use of β,β -carotene for the treatment of erythropoietic photoporphyrin has been questioned.⁶³²

Small-scale lutein/zeaxanthin supplementation studies support that consumption of food rich in dark green, leafy vegetables, and thus in eye xanthophylls, is associated with a substantially reduced risk of AMD in the elderly.⁵¹³ Similar conclusions have been obtained in a study of the effects of dietary intake of antioxidants, including β,β -carotene, in the risk of incident AMD.⁶³³ The LUXEA (lutein xanthophyll eye accumulation) study of xanthophyll accumulation in the human retina during supplementation with lutein or zeaxanthin indicated that the former carotenoid is deposited in the fovea, and the latter extends beyond the center of the macula, thus influencing not only AMD but also more peripheral diseases.⁶³⁴ Large RCTs by the American National Eye Institute directed to evaluate whether supplementation with either Zn or β,β -carotene/vitamin C/E mixtures (Age-related Eye Disease Studies 1, AREDS1) can influence the progression of AMD led to inconclusive results.

Finally, carotenemia or hypercarotenemia, although not dangerous as β,β -carotene is not toxic, can produce orangeing of the skin or carotenoderma.

14. SYNTHESIS OF CAROTENOIDS

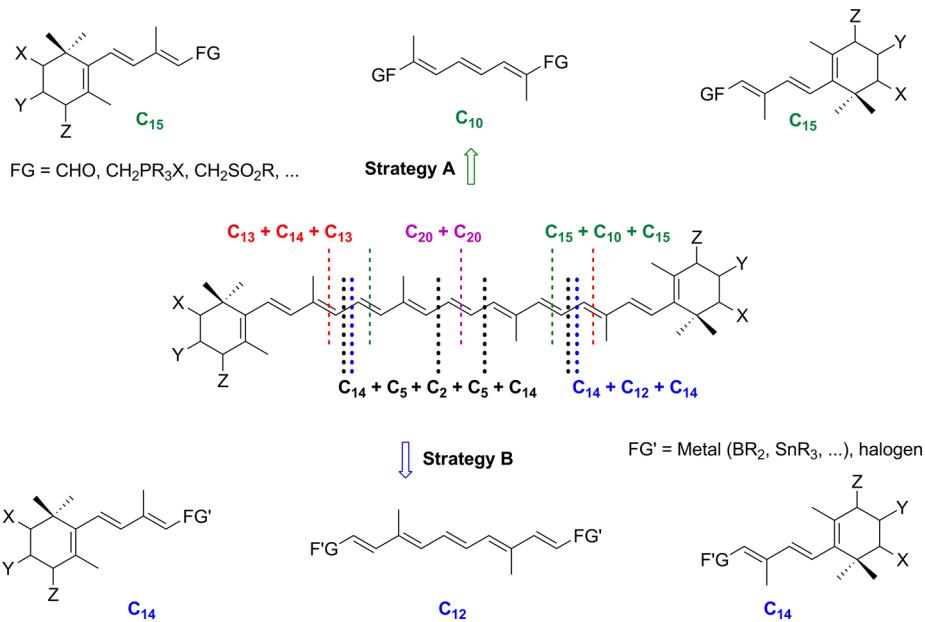
After optimization of the routes developed at Roche⁶³⁵ and BASF,⁶³⁶ β,β -carotene (**4**) has been produced commercially for use in the food industry (margarine, juice, health food-antioxidant, fertility cattle), providing about 40% of human dietary retinol activity equivalents (RAEs). Two major companies (BASF and DSM) produce about 85% of the

world supplies (more than 200 Tm per year). The production of β,β -carotene (**4**) from natural sources has become lately of commercial importance. The *Blakeslea trispora* fungal strain (also a source of lycopene, **481**), a soil bacteria of the *Sphingomonas* genus, and the marine algae *Dunaliella salina* grown in harvesting ponds are the most efficient alternatives for the manufacture of β,β -carotene (**4**).

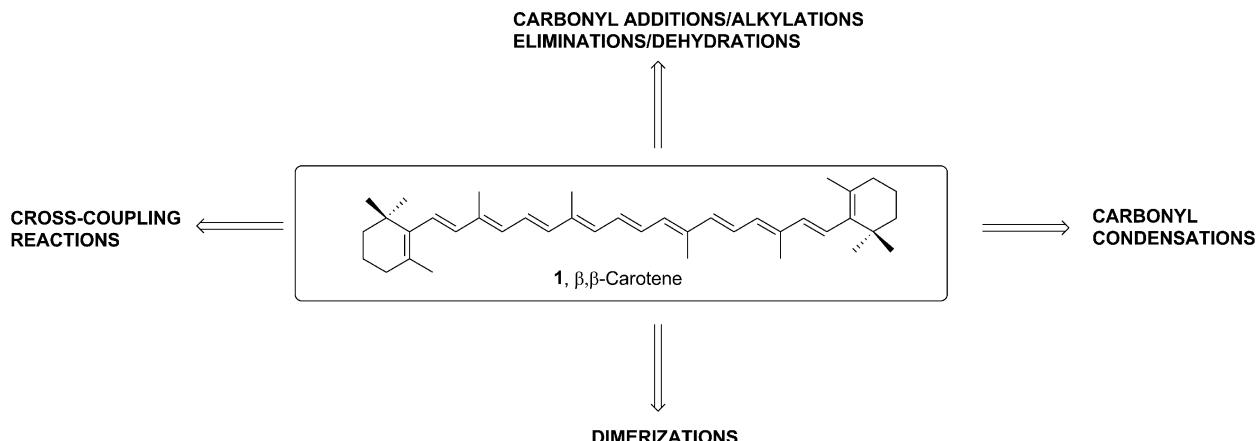
Five other carotenoids, two C₄₀ symmetrical carotenoids and three apocarotenoids (Figure 40), are also produced commercially in the nutraceutical industry as food products (vitamin supplements, feed additives for poultry, livestocks, fish, and crustaceans) and as cosmetics: C₃₀ 8'-apo- β -carotenal (**13**) (cheese, dressings),⁶³⁷ C₃₀ ethyl 8'-apo- β -carotenoate (**642**) (poultry-egg yolk and broiler skin pigmentation),^{637,638} C₃₃ citranaxanthin (**643**) (poultry-egg yolk pigmentation),^{637,639} C₄₀ canthaxanthin (**561**) (poultry),⁶⁴⁰ and a mixture of stereoisomers of C₄₀ astaxanthin (**644**) (aquaculture-salmonidae, crustaceae).⁶⁴¹ The synthesis of enantiopure (3S,3'S)-astaxanthin (**528**) has been developed at BASF.

Process syntheses of additional carotenoids of potential commercial value, C₄₀ lycopene (**581**),⁶⁴² zeaxanthin (**492**),⁶⁴³ and capsorubin (**633**),⁶⁴⁴ have also been developed, although the latter can be obtained from paprika extracts. Lutein (**491**) is commercially produced by extraction from saponified marigold flowers (*Tagetes erecta*).

BCC Research has estimated that the global market for carotenoids as antioxidants, vitamin precursors, natural colorants, and odorants was nearly \$1.2 billion in 2010 (\$261 million for β,β -carotene) and is expected to grow to \$1.4 billion

Scheme 65. General Strategies for C₄₀ Carotenoid Construction

Scheme 66. Overview of β,β-Carotene Retrosynthesis



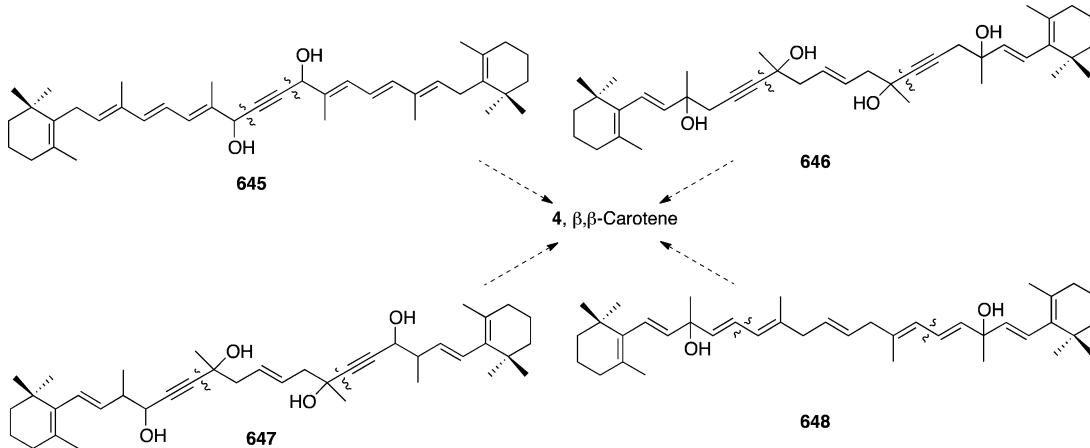
in 2018, thus becoming an important global market commodity.⁶⁴⁵

The syntheses of over 200 carotenoids have been reported.³³ An excellent description of the general methodological aspects can be found in an authoritative monograph.³³ Review articles have updated the synthetic efforts up to 2009 for general^{39b} and specific carotenoids.⁶⁴⁶ A report on xenobiotic carotenoids described the design of extravagant polyenes inspired in the structure of the natural products.⁶⁴⁷

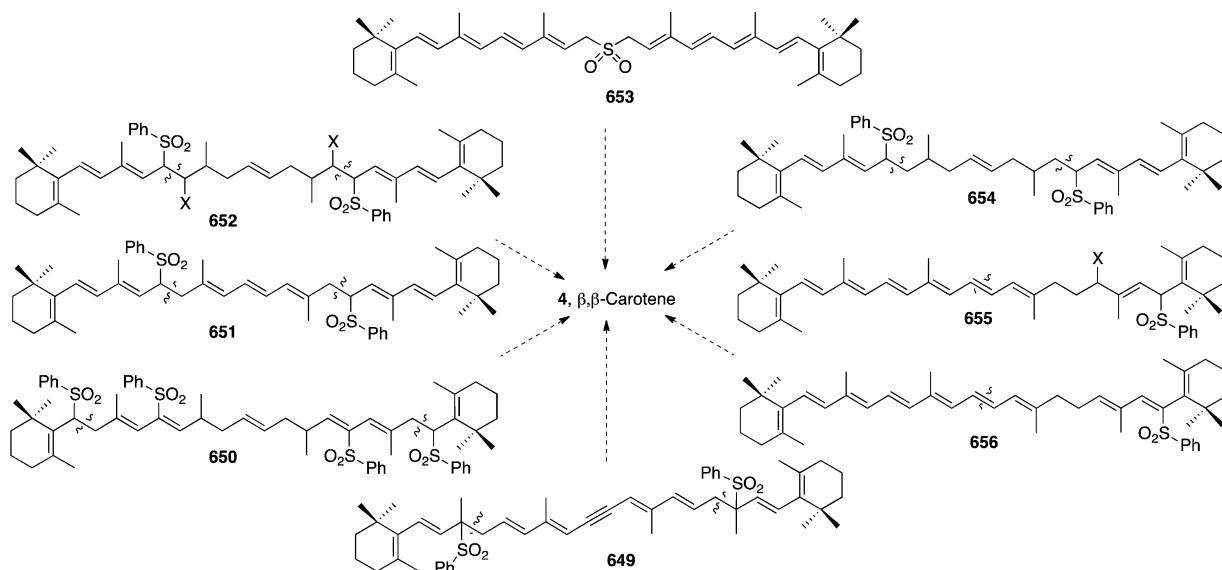
Similarly to retinoids, two general strategies are usually considered when approaching the construction of the carotenoid conjugated chain, and they differ in the nature of the bond between unsaturated carbon atoms that is formed in the key step. The C₂-symmetric structure of some of these polyene natural products makes symmetry criteria of critical importance for skeletal disconnections. Several disconnection schemes for a general C₄₀ carotenoid with modifications at the rings are shown in Scheme 65, and two of them are displayed to illustrate the strategies. Alternative tactics within each of these categories are described by the sum of the number of carbon atoms that the selected fragments contribute to the C₄₀ carotenoid skeleton.

In addition to their longer polyene skeletons, carotenoids are more complex than retinoids due to the diversity of end groups, the presence of oxygen substituents and stereogenic elements (centers, axes) at various positions, and the rearranged skeletons of some family members (norcarotenoids, <C₄₀). All of these factors contribute to the greater synthetic challenge associated with these polyenes when compared to the retinoids.

The main features of the bond-construction methodologies used in the different approaches to carotenoids have been discussed in detail in the retinoids section. Thus, we will summarize in general schemes (Schemes 66–71) selected polyene construction steps that have been used over the years for the preparation of the representative member, β,β-carotene (4). The classical strategies for the synthesis of most of the carotenoids will be discussed next, with a focus on methodological advances for oligoene construction. Finally, section 14.2.4 will summarize recent contributions to the synthesis of complex, highly functionalized xanthophyll norcarotenoids (C₃₇) with an emphasis on contemporary strategies to these compounds.

Scheme 67. Synthesis of β,β -Carotene (4) by Dehydration of Allyl and Propargyl Diols and Tetraols^a

^aWavy lines denote the previous skeletal-construction step.

Scheme 68. Synthesis of β,β -Carotene (4) by (Double) Sulfone Elimination and SO₂ Extrusion Reactions^a

^aWavy lines denote the previous skeletal-construction step.

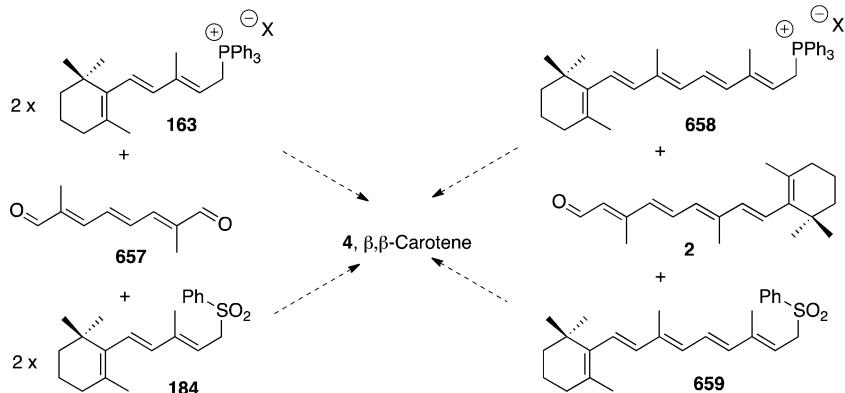
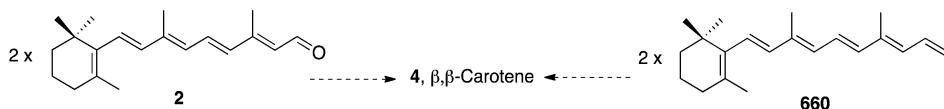
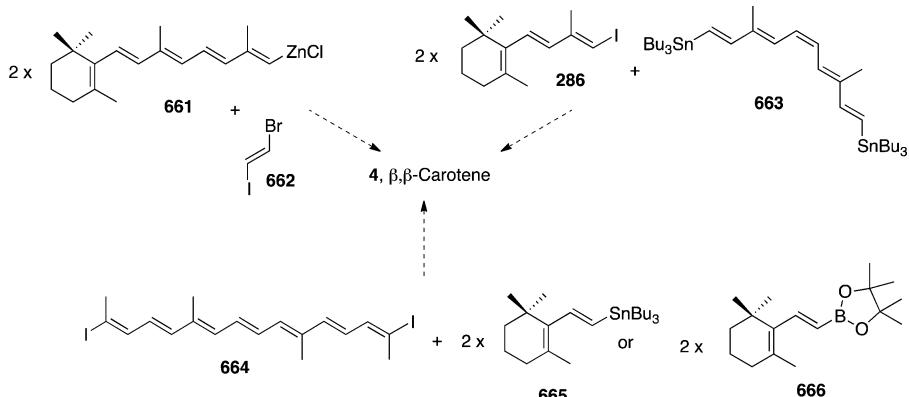
14.1. General Approaches to β,β -Carotene

Scheme 66 summarizes the general synthetic approaches to the β,β -carotene polyene skeleton from the corresponding precursors. Most of the methods have taken advantage of the C_2 -symmetric structure of β,β -carotene (4) and have used bidirectional tactics. In addition, methods for the direct dimerization of precursors have been adapted to the construction of these polyenes. Although the aldol condensation and its variants have provided several carotenoids (in particular those with a κ -end group and hemicyclic apocarotenoids),³³ the approaches to β,β -carotene (4) have proven inefficient and will not be covered here.

14.1.1. Construction of the Polyene by Dehydration or Elimination Reactions. The first synthesis of β,β -carotene (4) was reported independently by three groups. The three approaches (Scheme 67) were based on additions of acetylidyne anions to carbonyl compounds. This step was followed either by extended dehydration of 645 and hydrogenation of intermediate 15,15'-didehydro- β,β -carotene⁶³⁵ or by reduction of the propargylic alcohols to the allyl alcohols and dehydration

in the case of 646 and 647.⁶⁴⁸ A double dehydration of diol 648 obtained by Heck coupling of a C₁₅ allyl alcohol (172, Scheme 23) and a C₁₀ diiodide also generated β,β -carotene.⁶⁴⁹ A final isomerization to the *trans* isomer (4) was required in all cases shown in Scheme 67.

The elimination of phenylsulfinic acid from the substitution product of an allyl halide by an allyl phenyl sulfone has been another popular method for the generation of the carotenoid polyene skeleton (Scheme 68). Double elimination of the sulfone alkylation product 649 generated 15,15'-didehydro- β,β -carotene, which was hydrogenated and isomerized to 4 as explained before.⁶⁵⁰ Included within this group is the β,β -carotene synthesis developed by Büchi based on the formation of the bis-allylsulfone derived from all-*trans*-retinol (treatment of 1 with thiophthalimide in basic media and sulfinate-to-sulfone rearrangement to 653), and subsequent Ramberg-Bäcklund rearrangement of 653 after in situ formation of the α -halosulfone with *n*-BuLi and trapping with iodine or bromine.⁶⁵¹ Wittig reactions and sulfone chemistry have been combined using as a common building block the C₂₀

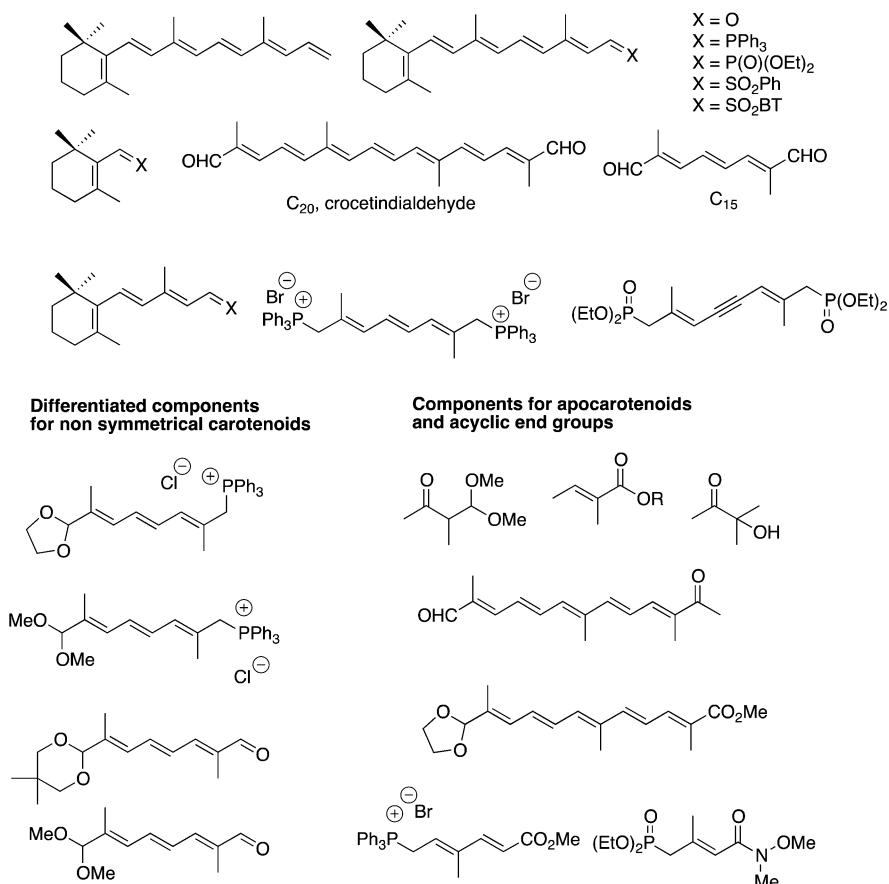
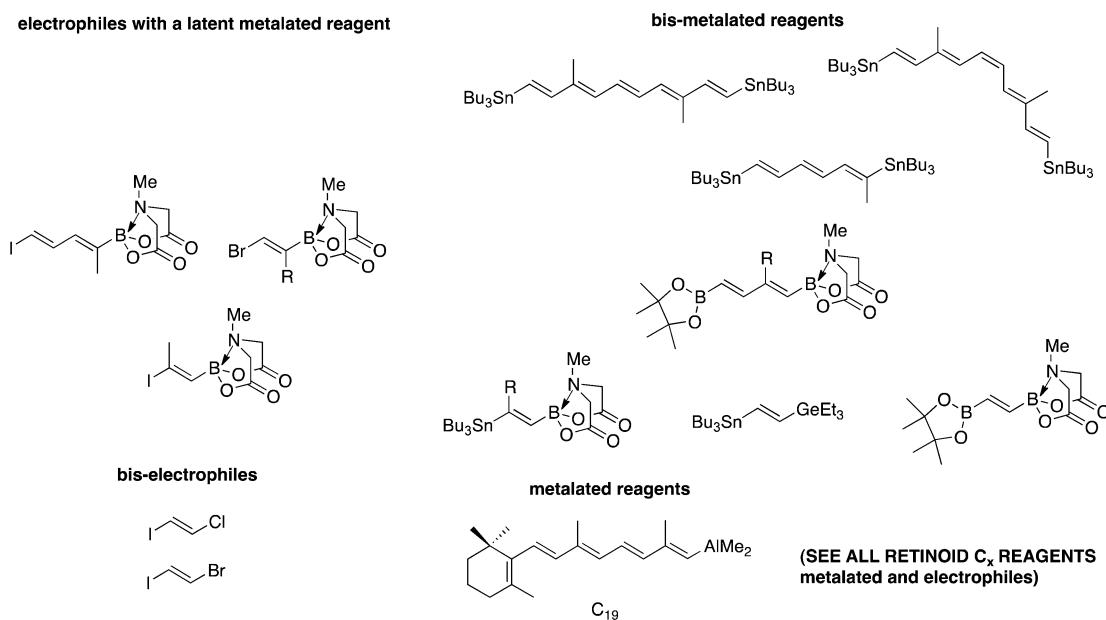
Scheme 69. Synthesis of β,β -Carotene (4) by Position-Selective Carbonyl Condensation Reactions (Wittig, Julia–Lythgoe)Scheme 70. Synthesis of β,β -Carotene (4) by Homodimerization ReactionsScheme 71. Synthesis of β,β -Carotene (4) by Pd-Catalyzed Cross-Coupling Reactions

phosphonium salt derived from retinol (see 658, Scheme 69) and C₂₀ aldehydes containing chloroallyl-, acetalalkyl-(X), and alkenyl-sulfone groups,⁶⁵² followed by double elimination processes induced by treatment of 655 and 656 with KOH or KOMe.⁶⁵² Other versions of the double sulfone alkylation/elimination approach to symmetrical carotenoids developed included as intermediates bis-allylsulfones 651⁶⁵³ and 654⁶⁵⁴ and a bis-alkyl, bis-vinyltetrasulfone 650.⁶⁵⁵ These intermediates were instead obtained by Ramberg–Bäcklund rearrangement of the precursors with an additional central bis-allylsulfone substructure obtained by alkylation of sulfones 184 (Scheme 19) and 198 (Scheme 21). Alternatively, a double elimination (KOMe, cyclohexane, 80 °C, 16 h) of the Julia adduct 652 formed by addition of the sulfone-stabilized anion of 184 (Scheme 19) to a γ,δ -unsaturated aldehyde and functionalization of the hydroxysulfone (halide, THP, EE, and MOM acetals, preferably the latter) was used in a variant of the Julia–Lythgoe reaction.⁶⁵⁶

14.1.2. Position-Selective Condensation of Carbonyl Compounds and Heteroatom-Stabilized Anions. The Wittig reaction was adapted at BASF for the synthesis of β,β -carotene (4) using the double condensation of C₁₀ dialdehyde 657 and two units of the C₁₅ phosphorane derived from 163, and alternatively by the reaction of all-trans-retinal (2) and the

anion of C₂₀ phosphonium salt 658 obtained from all-trans-retinol (1) (Scheme 69).⁶³⁶ A variant of the 2-fold Wittig approach involved the in situ formation of the phosphorane derived from 163⁶⁵⁷ using the reaction of π -allylpalladium complexes of allylic phenyl carbonates with a phosphine. Roche developed several syntheses of β,β -carotene (4) based on the Julia–Lythgoe condensation of sulfones and aldehydes.⁶⁵⁸ The 2-fold condensation of C₁₅ allylsulfone 184 and C₁₀ dialdehyde 657 following the Julia–Lythgoe protocol (addition of acetic anhydride to the β -hydroxysulfone and dithionite reductive elimination) produced the di-cis isomer, which was isomerized to β,β -carotene (4). The alternative Julia–Lythgoe condensation of two C₂₀ fragments (659 + 2) was a more direct route.⁶⁵⁸

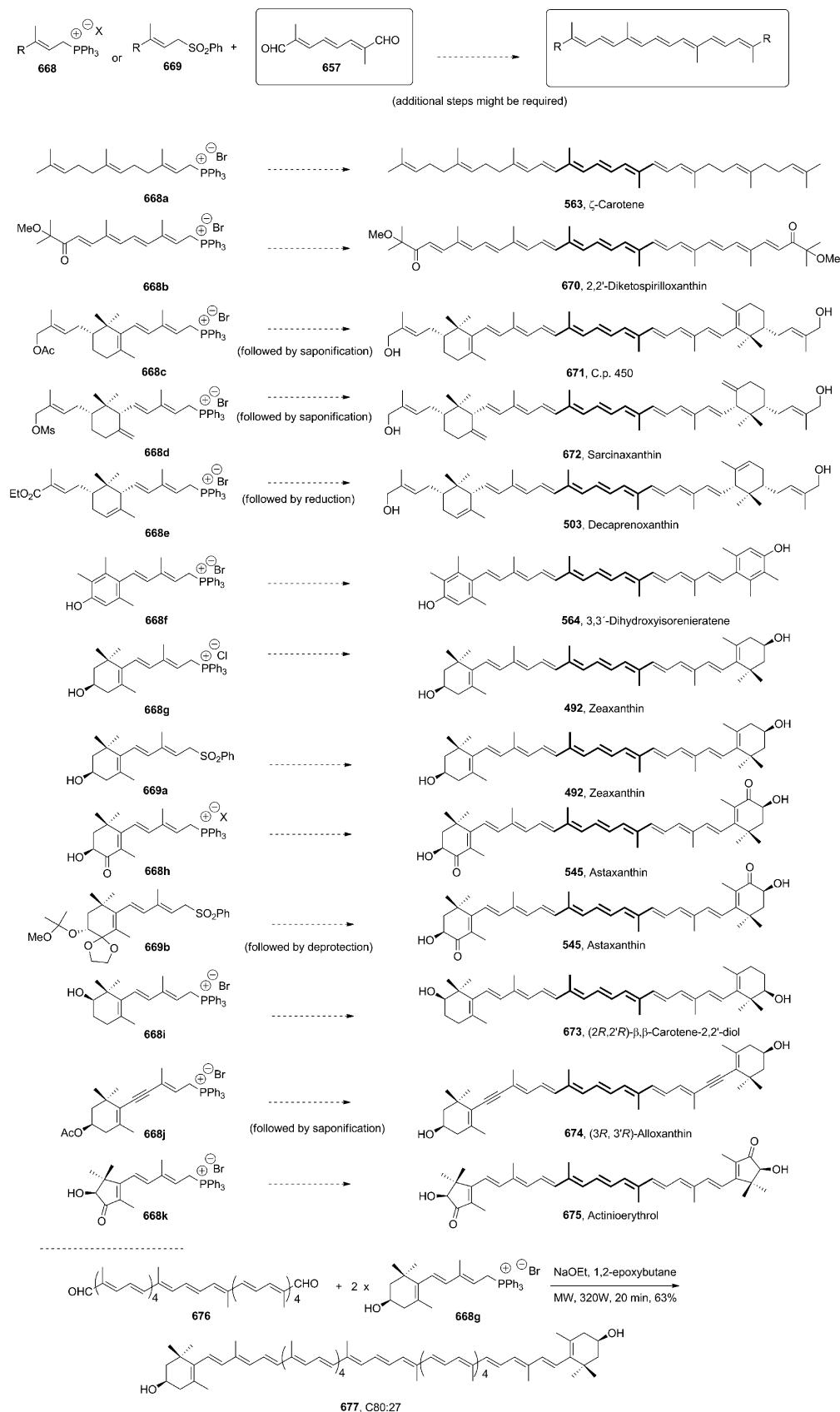
14.1.3. Homodimerization Reactions. The McMurry carbonyl coupling and the olefin metathesis reactions also exhibit positional selectivity in the double-bond construction step, although they have been less frequently used in polyene construction. McMurry's low valent titanium coupling of all-trans-retinal (2) generated β,β -carotene (4) in an excellent yield.⁶⁵⁹ The homometathesis/dimerization of hexaenes such as 660 (the Wittig product of all-trans-retinal 2) provided also a rapid entry into β,β -carotene (4) and other symmetrical carotenoids (Scheme 70).^{433b}

Symmetrical components for C₂-symmetric (and non symmetrical) carotenoidsFigure 41. The toolkit of reagents for the synthesis of carotenoids by position-selective Csp²=Csp² bond formation.Figure 42. The toolkit of mono- and bis-functionalized fragments for carotenoid Csp²-Csp² bond formation.14.1.4. Position-Selective Csp²-Csp² Cross-Coupling Reactions.

The first report on this approach to carotenoids employed the Negishi coupling of (*E*)-bromoiodoethene (**662**) with the C₁₉ organozinc reagent **661** obtained by trans-metathesis from the organoalane that results from the Zr-

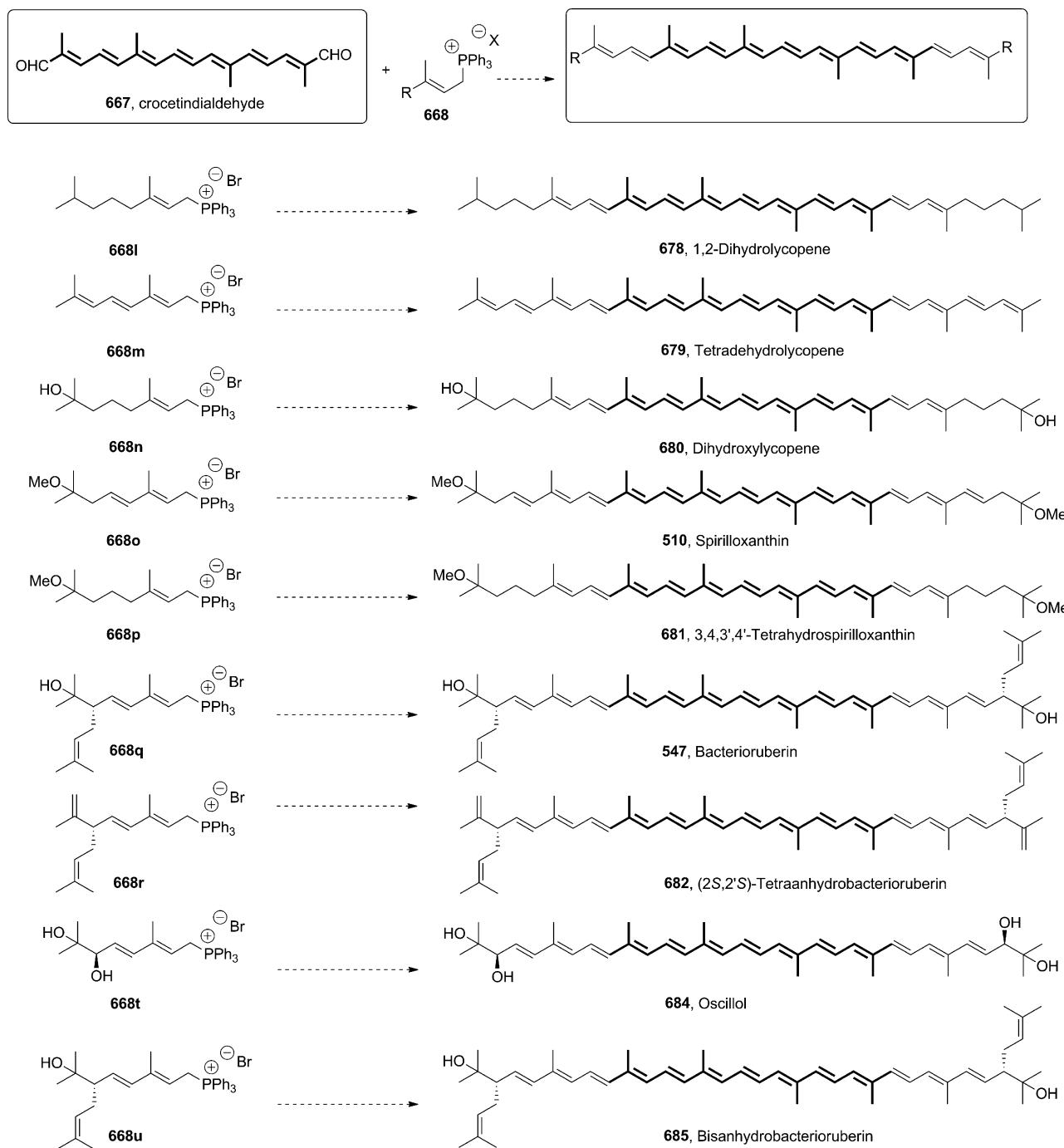
promoted methylalumination of precursor tetraenyne **386** (Scheme 46).⁴¹⁴ A two-directional Stille reaction of pentaeenylbis-stannane **663** and trienyl iodide **286** (Scheme 32) with concomitant isomerization of the central C15–C15' Z olefin was later reported (Scheme 71).⁶⁶⁰ A more extended

Scheme 72. Selected C_2 -Symmetric Carotenoids Prepared by Two-fold Wittig Condensation of C_{10} -Dialdehyde 657 with the Anions Derived from Phosphonium Salts 668 or Sulfones 669^a



^aIncluded is the preparation of carotenoid C80:27 (677).

Scheme 73. Symmetrical Carotenoids Prepared by Wittig Condensation of C₂₀-Dialdehyde 667 with the Phosphoranes Derived from Phosphonium Salts 668



heptaenylidiodide **664** was used as conjunctive C₁₈ reagent in 2-fold and bidirectional Stille and Suzuki coupling reactions with dienyl organometals **665** and **666**, respectively.⁷¹⁰

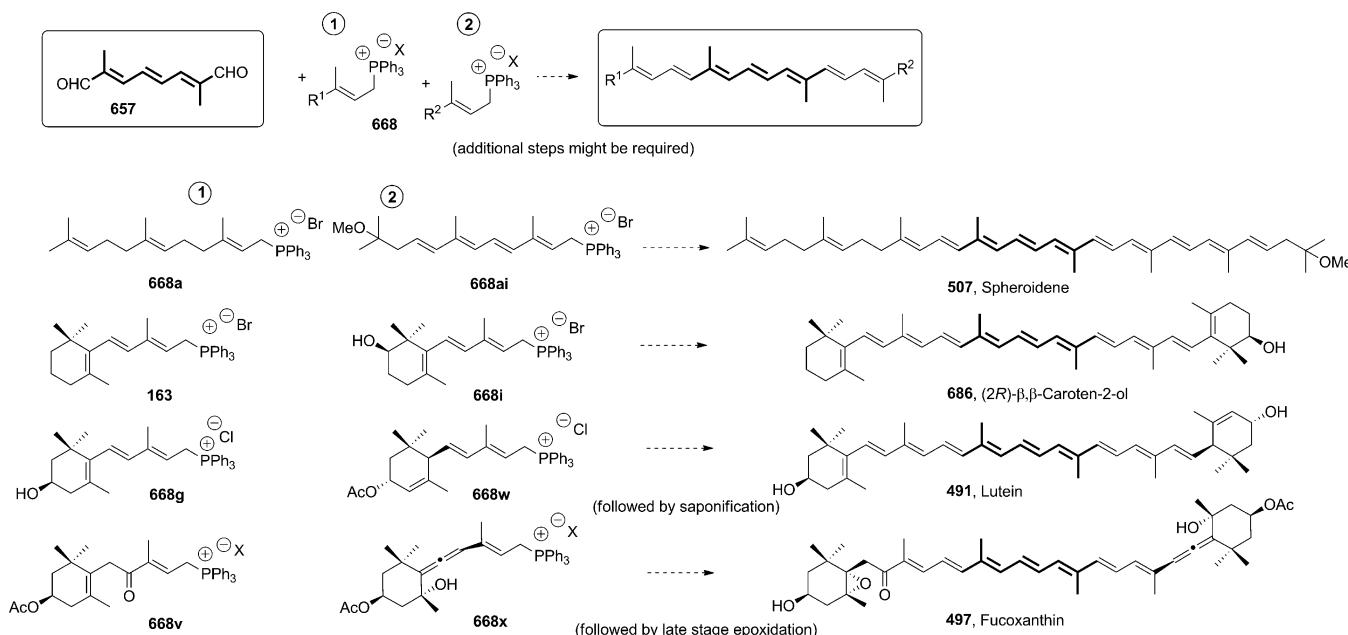
14.2. General Synthetic Approaches to Carotenoids

The synthetic approaches to carotenoids have taken full advantage of the central region oligoene skeleton being preserved intact in most of these natural products, including apocarotenoids and higher carotenoids, because in general the biogenetic modifications of the parent C₄₀ hydrocarbons are found at the ring carbons and/or at the proximal double bonds. Therefore, strategies that use difunctionalized central fragments have been most useful in carotenoid synthesis. Figure 41 (for

Csp²=Csp²) and Figure 42 (for Csp²–Csp²) depict the toolkit of reagents that has been used over the years for the synthesis of carotenoids by position-selective bond formation, as discussed previously for retinoids.

Because carotenoids are found in nature as the thermodynamically more stable *E* isomers (with the exception of 7,8-didehydrocarotenoids, which are found as the 9-*cis* isomers, and in-chain substituted cross-conjugated carotenals), the approaches to *cis* isomers will not be considered. General methods for the synthesis of *Z* isomers of carotenoids have been presented elsewhere.³³

Scheme 74. Nonsymmetrical Carotenoids Prepared by Sequential Condensations of C₁₀ Dialdehyde 657 and Two Different Phosphoranes Derived from the Phosphonium Salts 668 Indicated^a



^aCircled numbers denote the order of condensation steps.

14.2.1. Synthesis of Carotenoids by Position-Selective Double-Bond Forming Reactions. Figure 41 contains the most useful synthetic building blocks for the construction of the carotenoid skeleton by double-bond formation (Wittig, HWE, Julia–Lythgoe).

Two fragments stand out of the carotenoid toolbox for condensation reactions, the C₁₀ dialdehyde (2E,4E,6E)-2,7-dimethylocta-2,4,6-trienedal (657)⁶⁶¹ and the C₂₀ dialdehyde (2E,4E,6E,8E,10E,12E,14E)-2,6,11,15-tetramethylhexadeca-2,4,6,8,10,12,14-heptaenedal (667), crocetindialdehyde, itself a natural product obtained in small amount from *Capsicum annuum*).⁶⁶² Symmetrical carotenoids have been largely synthesized by 2-fold Wittig condensation of a phosphorane derived from phosphonium salt 668 and C₁₀ dialdehyde 657 (Scheme 72)⁶⁶¹ or C₂₀ dialdehyde 667 (Scheme 73). In some cases, the sulfone-stabilized anion derived from 669 (Scheme 72) has been alternatively used. On the other hand, nonsymmetrical carotenoids and apo-carotenoids have been made using doubly functionalized fragments, with differential reactivity (Schemes 78–81 and 84). Apocarotenoids have also been constructed using allylphosphonium salts conjugated with an electron-withdrawing group (ester, Weinreb amide,⁶⁶³ etc.), which can be transformed further along the sequence into aldehydes (Figure 41).

The 2-fold condensation of 657 and the appropriate phosphoranes derived from phosphonium salts 668 or the phenylsulfone-stabilized anions (from 669) was the key step in the preparation of acyclic carotenoids ξ-carotene (563)⁶⁶⁴ and 2,2'-diketospirilloxanthin (670),⁶⁶⁵ C₅₀-carotenoids C.p. 450 (671),⁶⁶⁶ sarcinaxanthin (672),⁶⁶⁷ and decaprenoxyanthin (503),⁶⁶⁸ the end-aromatic carotenoid 3,3'-dihydroxyisorenieratene (564),⁵⁴³ and several xanthophylls. Zeaxanthin (492) was technically obtained using Wittig^{643,669} and Julia procedures,^{669b} and astaxanthin (545) was produced by Wittig⁶⁷⁰ and Julia condensations⁶⁷¹ in an industrial scale. In the Julia case, the hydroxysulfone was functionalized and reduced with

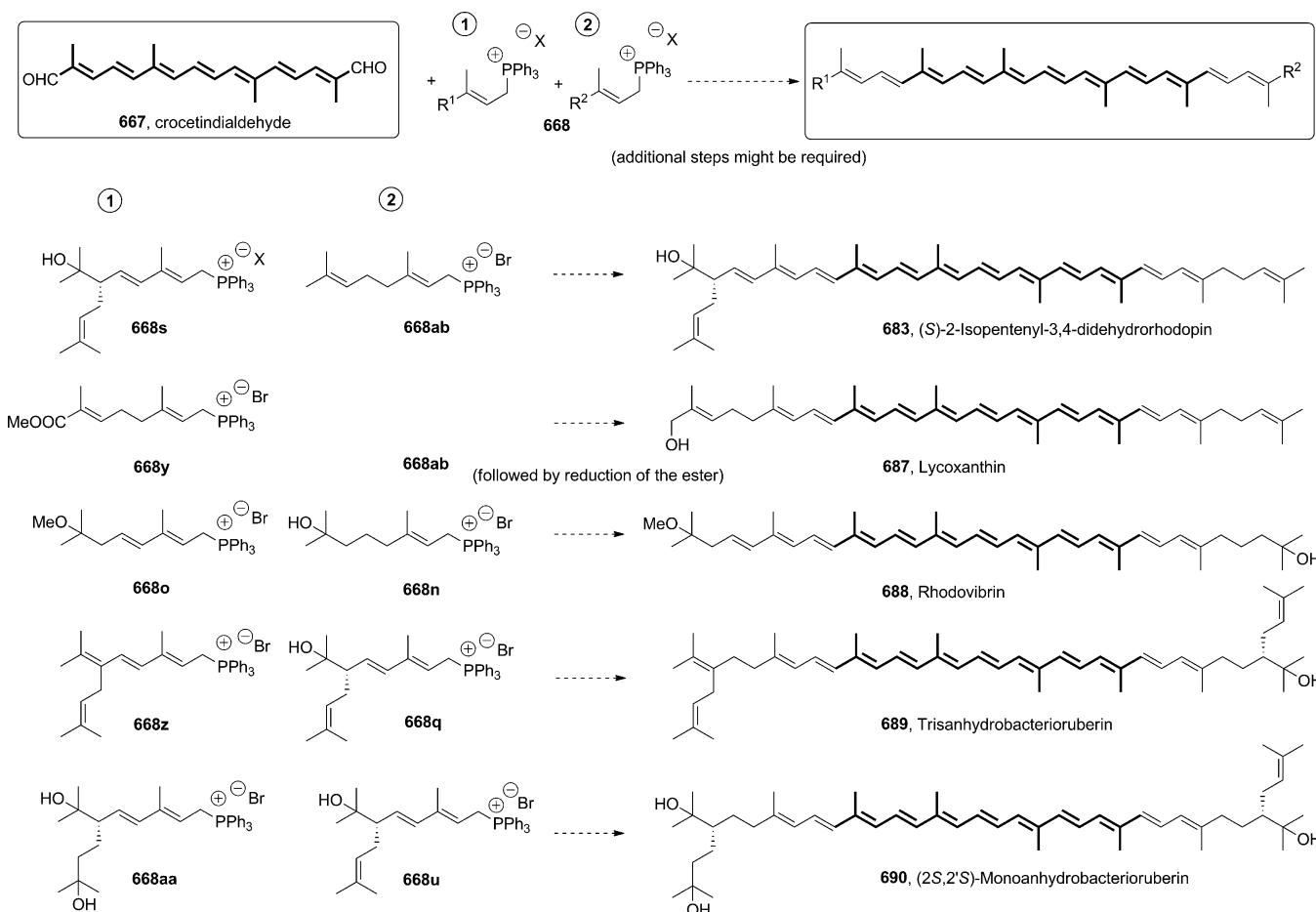
dithionite. (2R,2'R)-β,β-Carotene-2,2'-diol (673),⁶⁷² (3R,3'R)-alloxanthin (674) using alternatively triphenyl⁶⁷³ and tributylphosphoranes,⁶⁷⁴ and norcarotenoid actinioerythrol (675)⁶⁷⁵ followed the same condensation pattern.

An artificial carotenoid with 27 conjugated double bonds that showed an absorptium maximum at $\lambda_{\text{max}} \approx 527$ nm in CH₂Cl₂ (thus, a C80:27, compound 677, the longest polyene reported) was constructed by condensation of phosphonium salt 668 (same as for zeaxanthin but as a racemate) and C₅₀-dial 676 in the presence of NaOEt and 1,2-epoxybutane in EtOH under microwave irradiation conditions (320 W, 20 min, 63%).⁶⁷⁶

Crocetindialdehyde (667) has been a fundamental building block in the preparation of acyclic carotenoids, the majority of them of bacterial origin, including 1,2-dihydrolycopene (678),⁶⁷⁷ tetrahydrolycopene (679),⁶⁷⁸ dihydrolycopene (680),⁶⁷⁹ spirilloxanthin (510),⁶⁷⁹ 3,4,3',4'-tetrahydrospirilloxanthin (681),⁶⁷⁹ bacterioruberin (547),⁶⁸⁰ (2S,2'S)-tetrahydropacterioruberin (682),⁶⁷⁹ oscillo (684),⁶⁸¹ and bisanhydrotacterioruberin (685).⁶⁸² The aldol condensation of the same dialdehyde (667) was instead employed for the synthesis of cantaxanthin (561)⁶⁵⁸ and capsorubin (633).⁶⁴⁴

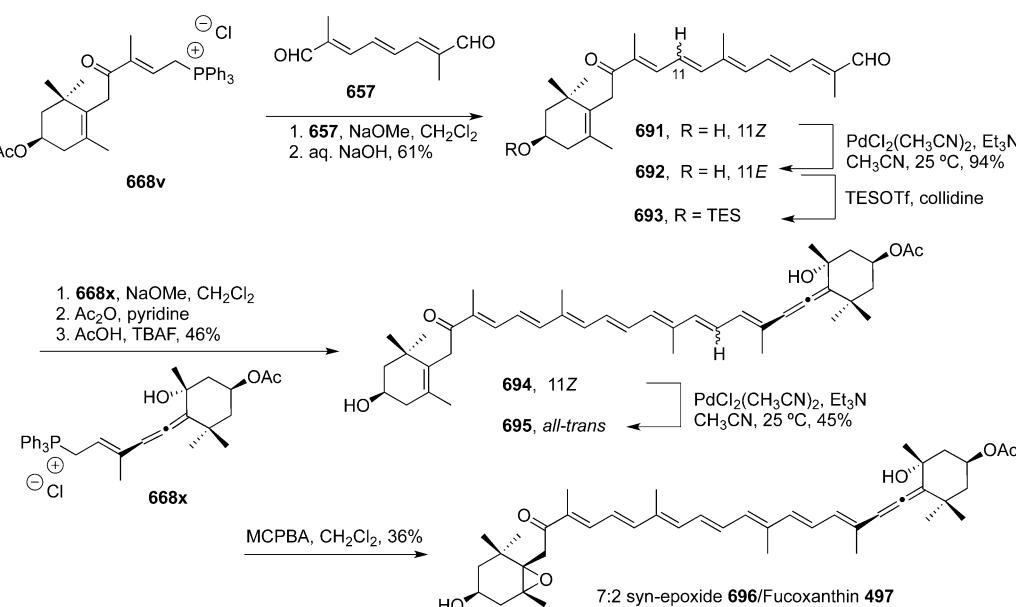
Using excess dialdehydes, monocondensation reactions with phosphoranes are feasible due to the lower reactivity of the apocarotenal formed in the first step. Thus, consecutive Wittig reactions of excess C₁₀ and C₂₀ dialdehydes (657 and 667) and two different phosphoranes can be employed, and the same building blocks can be alternatively used for the preparation of C₂-symmetric and nonsymmetrical carotenoids. The main limitations of this variant are the low yields and the poor stereoselectivity of the reaction, because mixtures of all-trans and di-cis isomers are in practice obtained. Included in this tactic are the preparation of acyclic spheroidene (507),^{665,683} (2R)-β,β-carotene-2-ol (686),⁶⁸⁴ lutein (491),⁶⁸⁵ and fucoxanthin (497),⁶⁸⁶ from C₁₀ dialdehyde 657 (Scheme 74), of (S)-2-isopentenyl-3,4-didehydrorhodopin (683),^{680a,b} of lycoxanthin (687),⁶⁸⁷ rhodovibrin (688),⁶⁸⁸ trisanhydrobacterioruber-

Scheme 75. Nonsymmetrical Carotenoids Prepared by Sequential Condensation of C₂₀ Dialdehyde 667 and Two Different Phosphoranes Derived from Phosphonium Salts 668^a



^aCircled numbers denote the order of condensation steps.

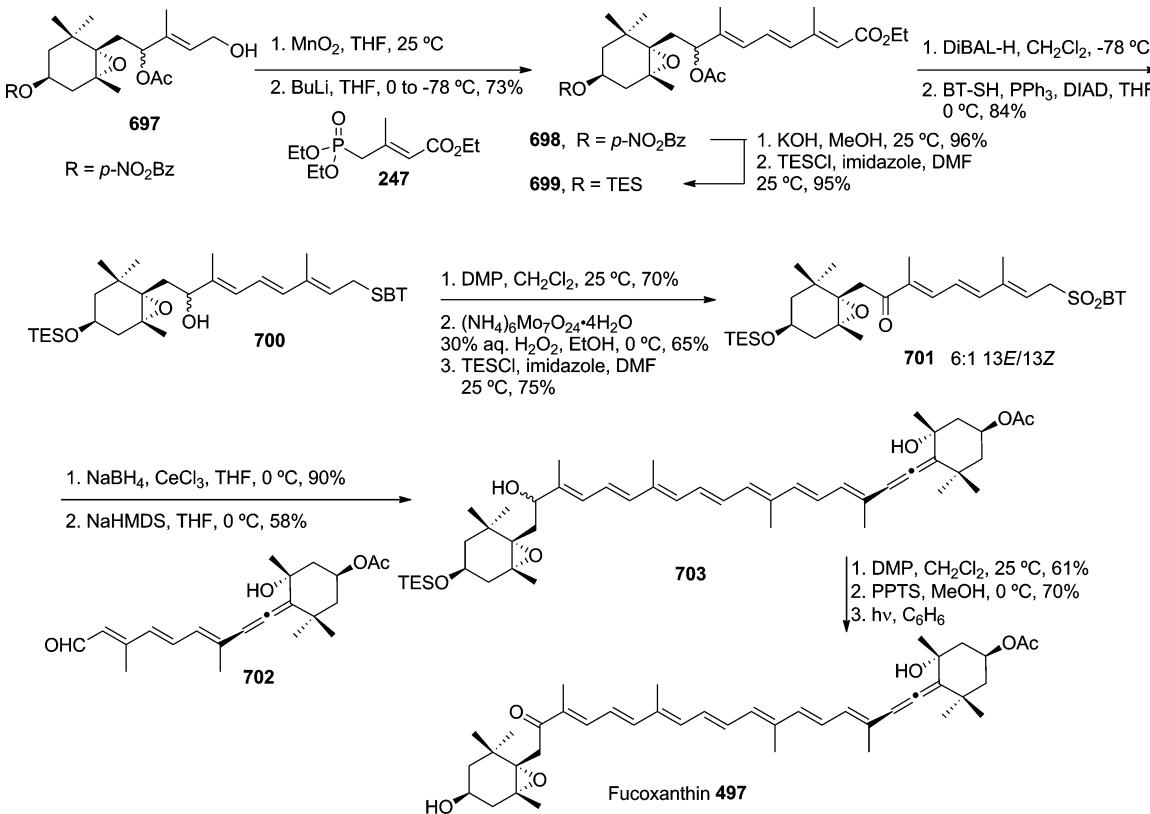
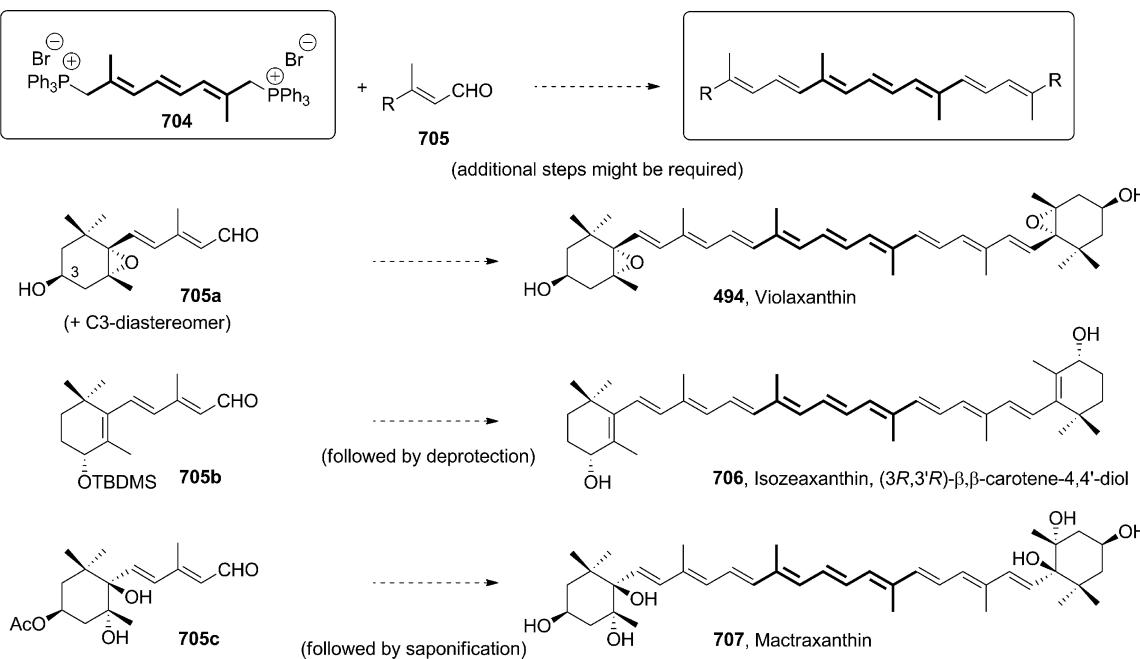
Scheme 76. Synthesis of Fucoxanthin (497) by Sequential Condensations of C₂₀ Dialdehyde 657 with Two Different Phosphoranes



in (689),⁶⁸⁹ and (2S,2'S)-monoanhydrobacterioruberin (690)⁶⁸⁹ from C₂₀ dialdehyde 667 (Scheme 75).

As an example of the sequential Wittig chemistry for the synthesis of nonsymmetrical carotenoids, the preparation of

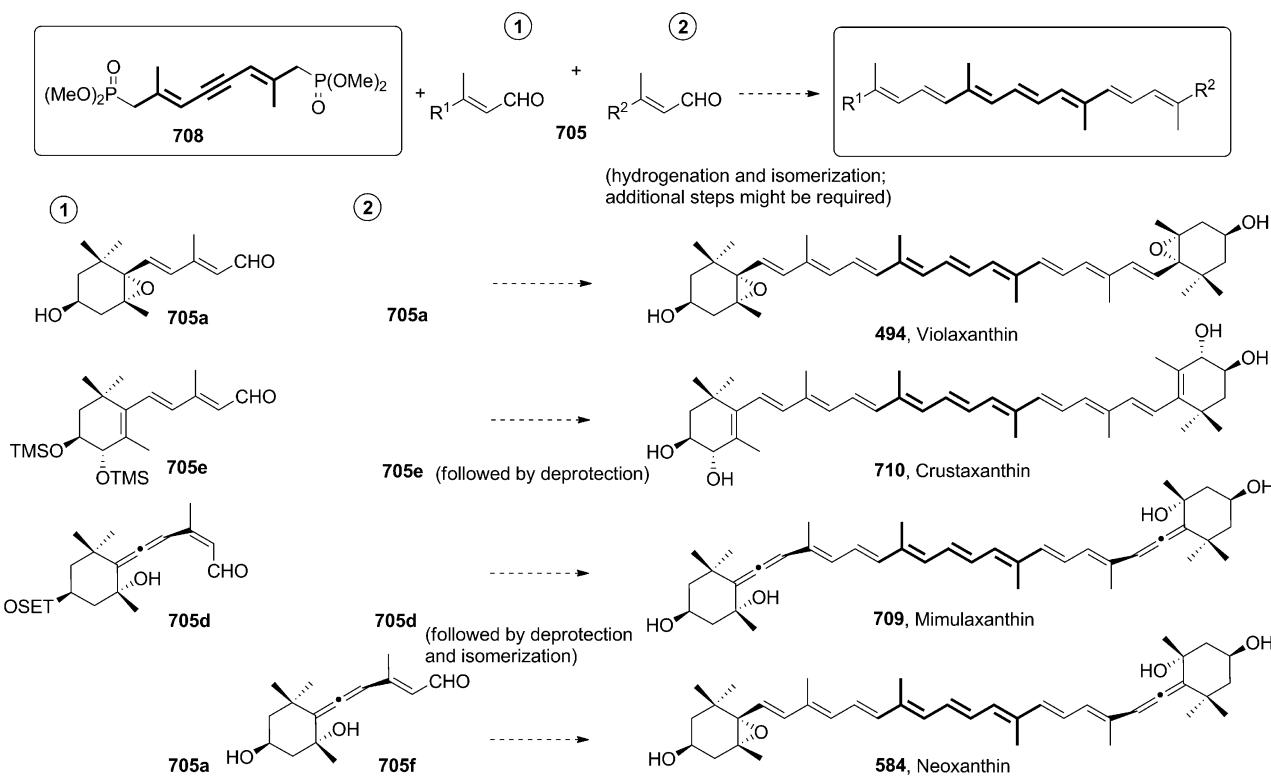
Scheme 77. Synthesis of Fucoxanthin (497) by Julia–Kocienski Condensation

Scheme 78. Synthesis of Symmetrical Carotenoids by Two-fold Condensations of C₁₀-Bisphosphonium Salt 704 with Aldehydes 705

fucoxanthin (**497**) is detailed in Scheme 76. The condensation of phosphonium salt **668v**, treated with NaOMe, and dialdehyde **657**, followed by saponification afforded in 61% yield a 1:1 mixture of 11*E*/11*Z* aldehydes **691**/**692**, which was isomerized to the all-*trans* isomer **692** after stirring with a Pd(II) complex [$\text{PdCl}_2(\text{CH}_3\text{CN})_2$], and the latter was

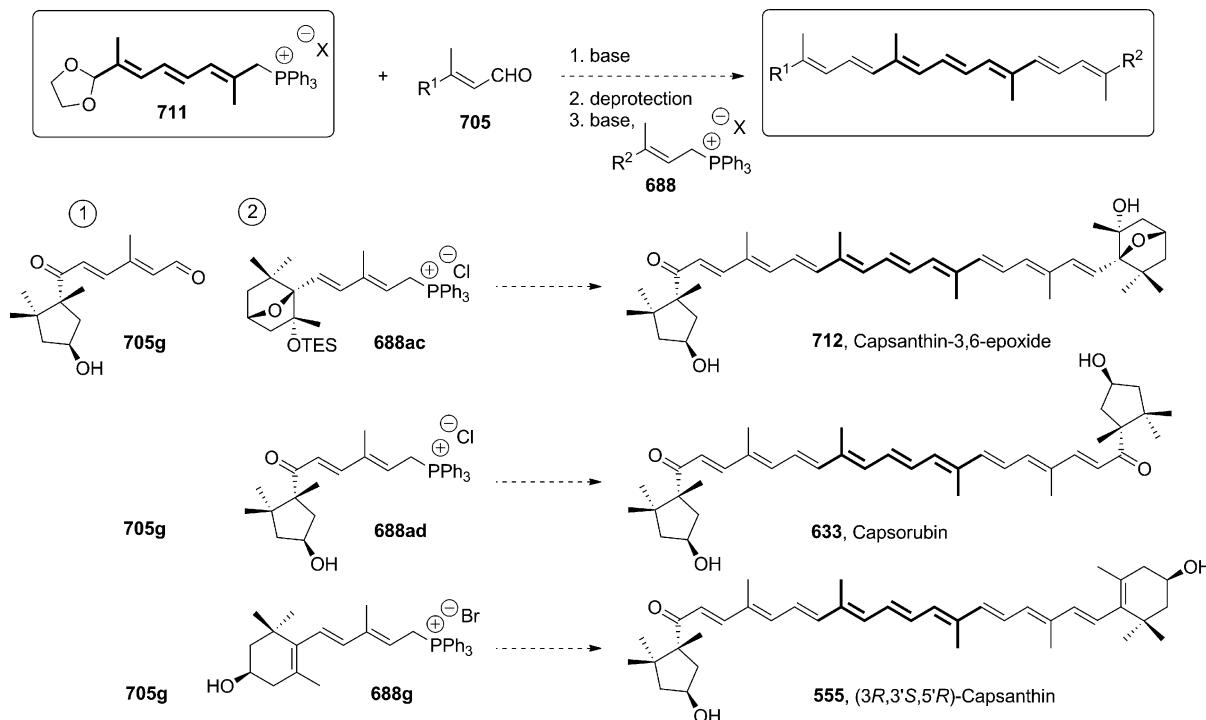
protected as the silyl ether **693**. The second Wittig condensation using **668x** also led to a similar mixture of isomers at the newly formed double bond. The product resulting from acetylation and deprotection was treated with a Pd(II) complex to induce isomerization and gave the *trans* isomer **695**. A final epoxidation of **695** using MCPBA took

Scheme 79. Synthesis of Symmetrical and Nonsymmetrical Carotenoids by Two-fold or Sequential Condensations of C₁₀-Bisphosphonate 708 with Aldehydes 705^a



^aTwo different aldehydes for neoxanthin 584; circled numbers denote the order of condensation steps.

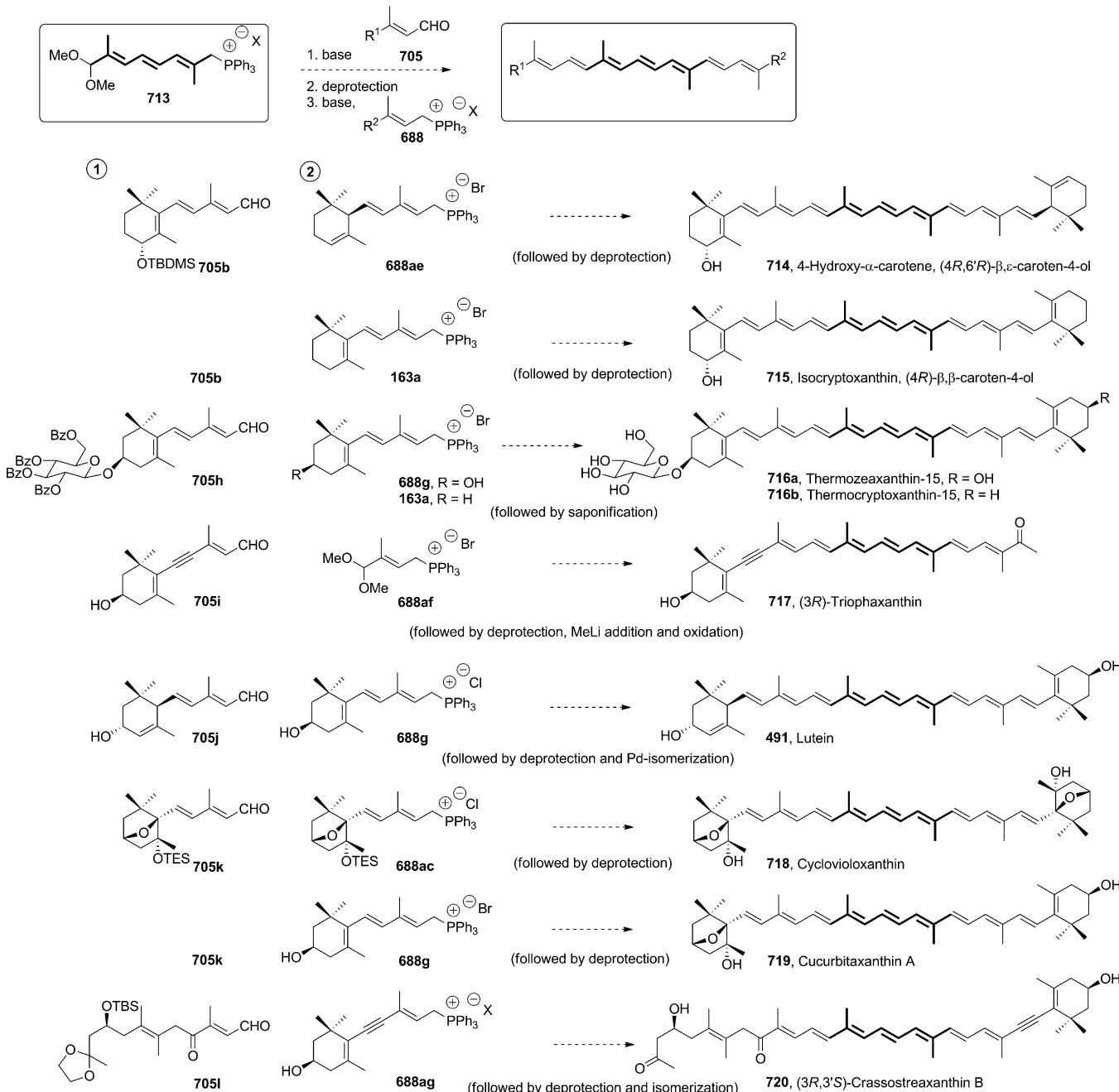
Scheme 80. Synthesis of Nonsymmetrical Carotenoids by Sequential Condensations of C₁₀ Acetal Phosphonium Salt 711^a



^aCircled numbers denote the order of condensation steps.

place in poor yield and with the undesired stereoselectivity, as the major product was the *syn* epoxide **696** and fucoxanthin (**497**) was the minor component (7:2 ratio).⁶⁹⁰

A more stereoselective approach to the same carotenoid was based on a final Julia–Kocienski reaction^{368a} of benzothiazolyl (BT)-sulfone **701** and aldehyde **702**.^{368b,691} The synthesis of

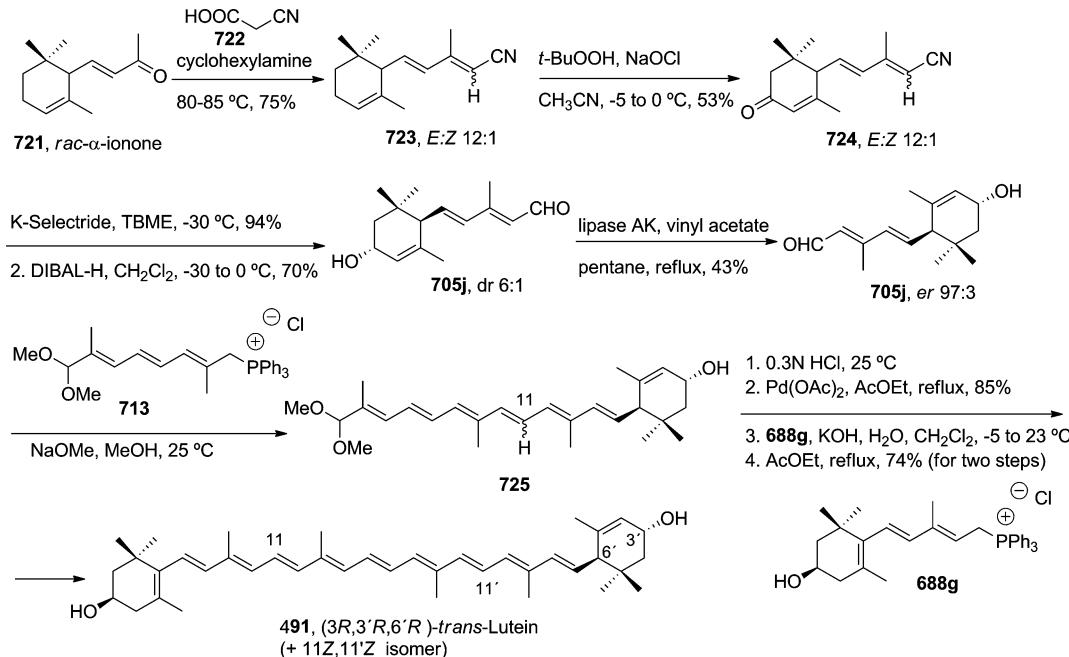
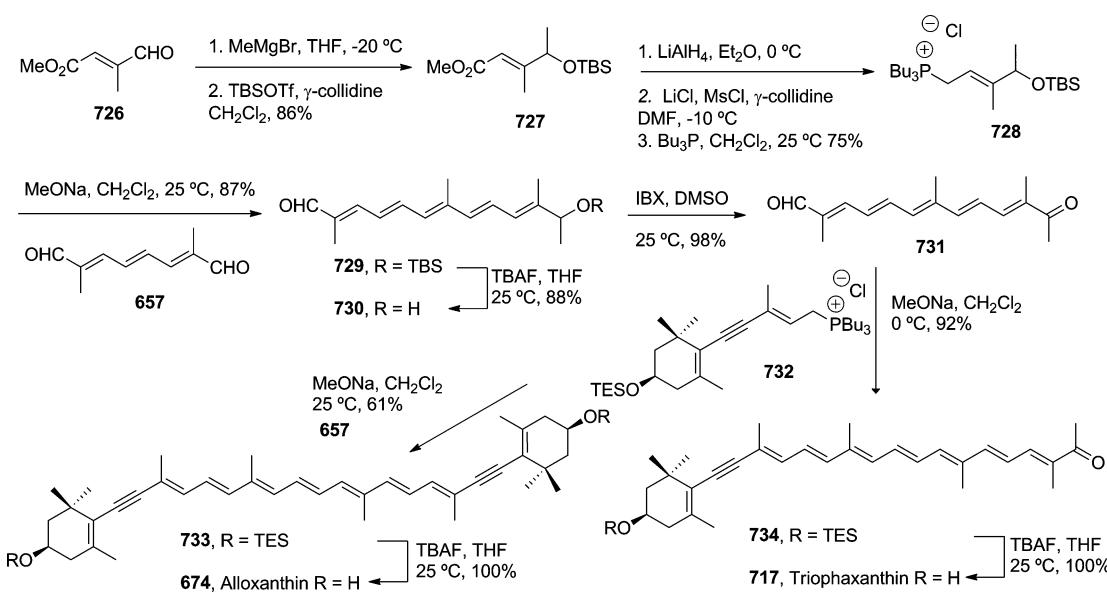
Scheme 81. Synthesis of Nonsymmetrical Carotenoids by Sequential Condensations of C₁₀ Acetal Phosphonium Salt 713^a

^aCircled numbers denote the order of condensation steps.

the former was carried out starting from *epi*-actinol, which was converted into the *p*-nitrobenzoate 697 by Mitsunobu inversion after a successful *syn*-directed epoxidation of an organoaluminum peroxide intermediate obtained from TBHP/Al(Ot-Bu)₃. HWE condensation of 247 with the aldehyde derived from 697 led to trienoate 698. Exchange of protective group at the secondary alcohol was followed by reduction and transformation of the primary allyl alcohol into the sulfide 700. Sequential oxidation of the secondary allylic alcohol to the trienone and of the sulfide to the sulfone (which also produced a minor amount of the double-bond isomer), and reprotection of the alcohol as TES derivative provided 701. The reduction of the ketone afforded the Julia–Kocienski BT-sulfone component. Reaction of the anion of this sulfone, generated by

treatment of the mixture of alcohols with NaHMDS at 0 °C in THF, and 702 under Barbier conditions afforded product 703 as a mixture of isomers in 56% yield. A new oxidation of the allyl alcohol with Dess–Martin periodinane (61%) and release of the silyl group (PPTS, 70%) provided fucoxanthin (497) as a mixture of isomers, which was enriched in the all-*trans* (76% isomeric ratio) by irradiation in benzene with a fluorescent light under argon for 3 days (Scheme 77).⁶⁹¹

In some cases, it has proven advantageous to exchange the functionalities of the partners and use a C₁₀ bisphosphonium salt such as 704. The requirement to eliminate triphenylphosphine oxide at the monoylide stage, however, limits its application for nonsymmetrical carotenoids. Some of the carotenoids prepared using the condensation of this building

Scheme 82. Synthesis of (*3R,3'R,6'R*)-All-*trans*-lutein (491)Scheme 83. Synthesis of Alloxanthin (674) and Triophaxanthin (717) Using Wittig Reactions with Tri-*n*-butylphosphonium Salts

block with aldehydes 705 are violaxanthin (494),⁶⁹² isozeaxanthin (706),⁶⁹³ and mactraxanthin (707).⁶⁹⁴

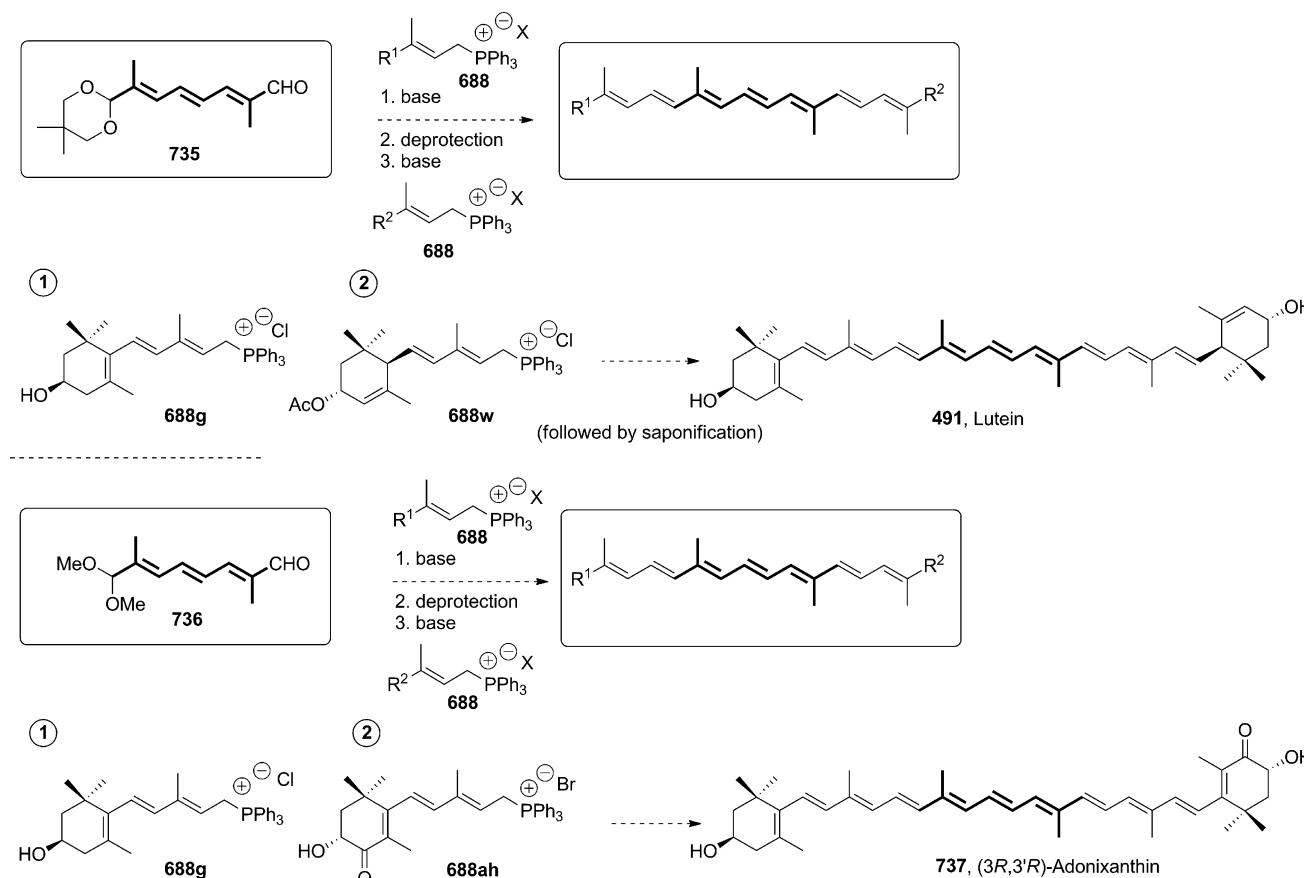
If the C₁₀ bisphosphonate 708 is used instead of the phosphonium salt 704, the resulting alkyne is hydrogenated to the *cis* isomer, and the sequence ends with the isomerization to the *trans* isomer (Scheme 79). Violaxanthin (494),⁶⁹⁵ mimulaxanthin (709),⁶⁹⁶ crustaxanthin (710),⁶⁹⁷ and the nonsymmetrical neoxanthin (584)⁶⁹⁵ have been made with this tactic.

Alternatively, central reagents with differentiated end groups, which have a protected aldehyde at one terminus and a phosphonium salt at the other, allow one to perform conjunctive Wittig chemistry iteratively (Schemes 80 and 81). The application of this tactic has led to the preparation of

nonsymmetrical carotenoids, such as capsorubin (633),⁶⁹⁸ capsanthin-3,6-epoxide (712),⁶⁹⁹ and (3*R,3'S,5'R*)-capsanthin (555)⁶⁹⁸ using the dioxolane phosphonium salt 711 (Scheme 80), and of 4-hydroxy- α -carotene (714),⁶⁷² isocryptoxanthin (715),⁶⁷² thermozeaxanthin-15 (716a) and thermocryptoxanthin-15 (716b),⁷⁰⁰ (3*R*)-triophaxanthin (717),⁷⁰¹ cucurbitaxanthin A (719),⁶⁹⁹ cyclovioloxanthin (718),⁶⁹⁹ (3*R,3'S*)-crassostreaxanthin B (720),⁷⁰² and lutein (491),⁷⁰³ using the dimethylacetal Wittig salt 713 (Scheme 81).

The use of these C₁₀ compounds with a protected aldehyde at one end and a phosphonium salt at the other circumvents the need to form an allylic phosphonium salts at one of the terminal positions of the carotenoid fragment.

Scheme 84. Synthesis of Nonsymmetrical Carotenoids by Sequential Condensations of Monoprotected C₁₀ Dialdehydes 735 and 736^a



^aCircled numbers denote the order of condensation steps.

The first total synthesis of (3*R*,3'*R*,6*R*)-lutein (**491**) with an overall yield of 1% was reported in 1980.⁶⁸⁵ A new synthesis of (3*R*,3'*R*,6*R*)-lutein (**491**) (Scheme 82) and its stereoisomers has been recently developed on the basis of the use of C₁₀ reagent **713**.⁷⁰³ For the (3*R*,3'*R*,6*R*) enantiomer, the sequence started with the Knoevenagel condensation of α -ionone (**721**) with cyanoacetic acid (**722**) in the presence of cyclohexylamine, which provided nitrile **723** as a mixture of double-bond isomers. Allylic oxidation at the C3 position to give **724** was achieved with *t*-BuOOH and NaOCl. Stepwise reduction of the ketone and nitrile groups by treatment first with K-Selectride and then with DIBAL-H afforded a mixture of the hydroxylaldehyde diastereomers in a 6:1 ratio where the (3*R*^{*},6*R*^{*}) predominated. The two enantiomers of the major diastereomer could be separated by enantiospecific acylation with vinylacetate and lipase AK from *Pseudomonas fluorescens* in refluxing pentane. After 48 h at 43% conversion, (3*R*,6*R*)-**705j** was obtained with an enantiomeric excess of 94%.⁷⁰³

Reaction of **705j** with the anion of phosphonium salt **713** provided **725** as a mixture of 11*E*/11*Z* geometric isomers. Hydrolysis of the acetal group and isomerization by treatment with Pd(OAc)₂ in EtOAc gave a major aldehyde of *trans* geometry, which was subjected to a second Wittig reaction with phosphonium salt **688g** and the mixture of double-bond isomers thermally isomerized by heating in EtOAc for 4 h, to give as major diastereomer (99:1 ratio) *trans*-**491** in 74% yield.⁷⁰³

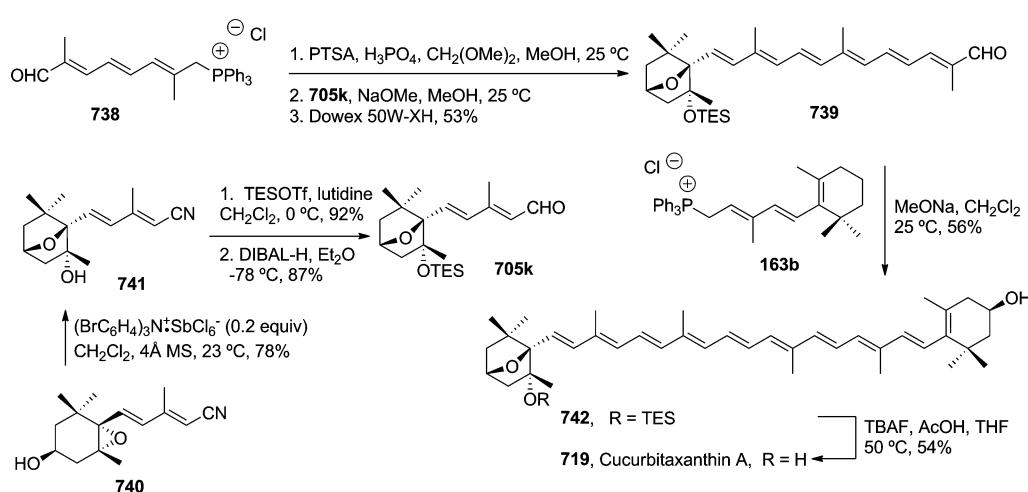
Similar sequences using the diastereomers of aldehyde **705j** produced from nitrile ketone **724** by reduction using Corey's CBS and epimerizations generated the remaining diastereomers.⁷⁰³

A recent synthesis of acetylenic carotenoids alloxanthin (**674**) and apocarotenoid triophaxanthin (**717**) used tributylphosphonium salts in two Wittig reactions (Scheme 83). The first involved the monocondensation of C₁₀ dialdehyde **657** and the allylphosphorane derived from phosphonium salt **728**.⁶⁷⁴ The deprotection and oxidation of alcohol **730** with IBX gave the C₁₆ dicarbonyl compound **731**. Selective condensation of the aldehyde with C₁₅ butylphosphonium salt **732** was possible if the temperature did not exceed 0 °C, and provided ketone **734**, which was efficiently deprotected to triophaxanthin (**717**). On the other hand, 2-fold condensation of the same Wittig salt with dialdehyde **657** and deprotection of the bis-acetylenic compound **733** furnished alloxanthin (**674**).

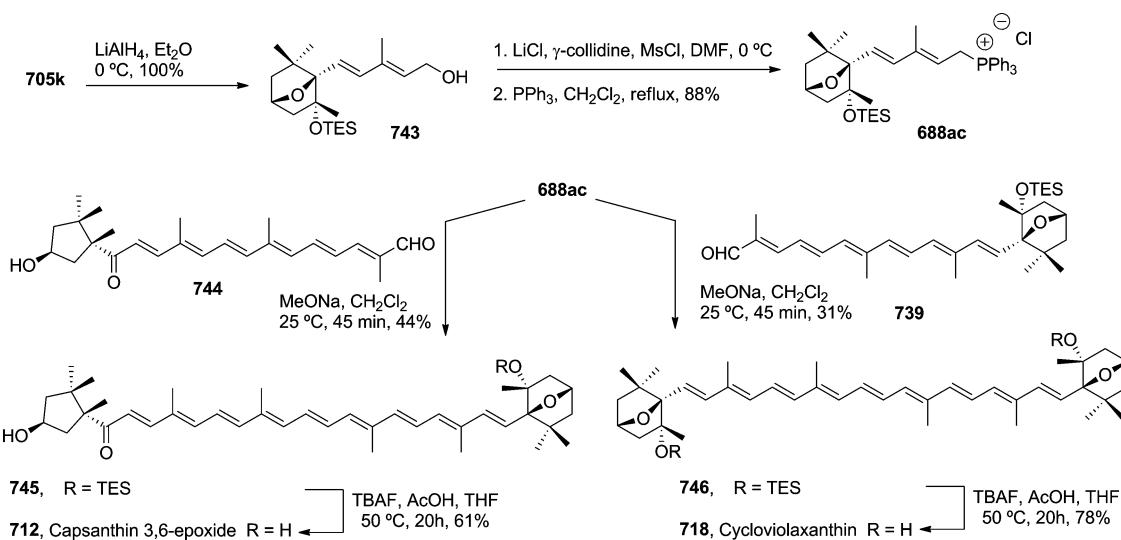
To favor monocondensation reactions of the dicarbonyl compounds with phosphonium salts, carotenoid building blocks with one of the aldehydes protected as acetal (C₁₀ compounds **735** and **736**, Scheme 84) have been used.⁷⁰⁴ Acetal cleavage then releases the second aldehyde for a subsequent condensation. This approach has been used in the synthesis of lutein (**491**)⁶⁸⁵ and 3-hydroxy-4-oxocarotenoids such as (3*R*,3'*R*)-adonixanthin (**737**).⁷⁰⁴

In some cases, the protection of the aldehyde has been effected prior to formation of the phosphorane and reaction with the aldehyde. Fragment **738** was used as central linchpin

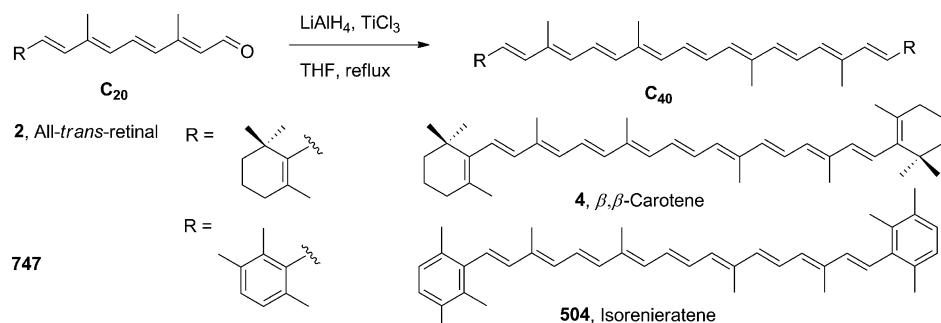
Scheme 85. Synthesis of Cucurbitaxanthin A (719) Using Wittig Reactions with *in Situ* Protection of a C₁₀-Aldehyde Phosphorane 738



Scheme 86. Synthesis of Capsanthin 3,6-Epoxyde (712) and Cycloviolaxanthin (718) Using Wittig Reactions



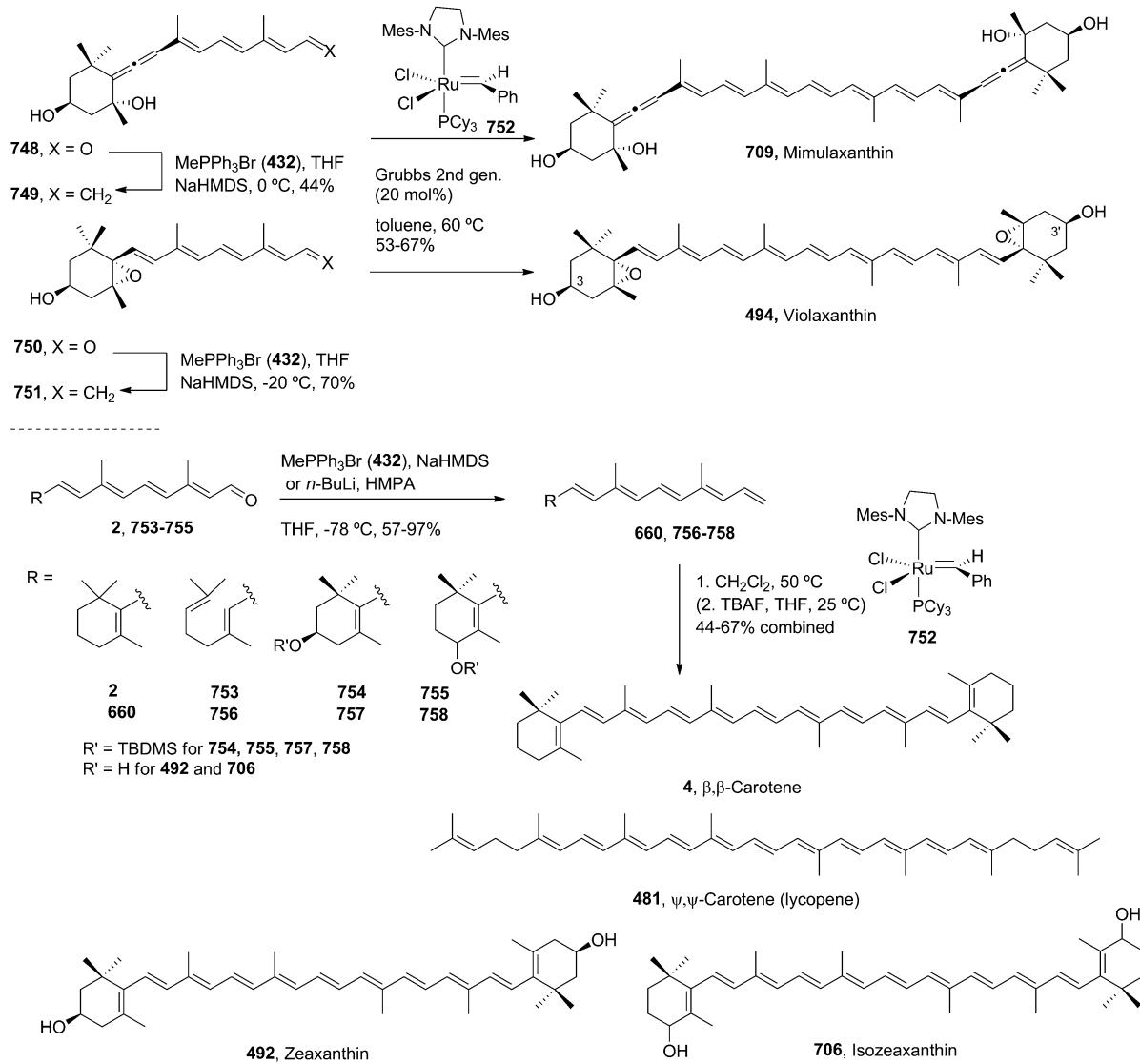
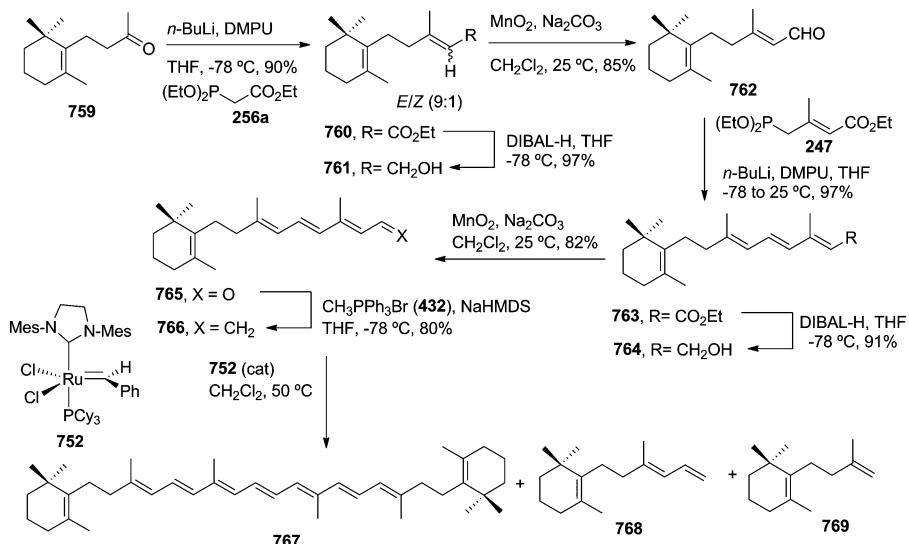
Scheme 87. Synthesis of C₄₀ Carotenoids by McMurry Carbonyl Dimerization



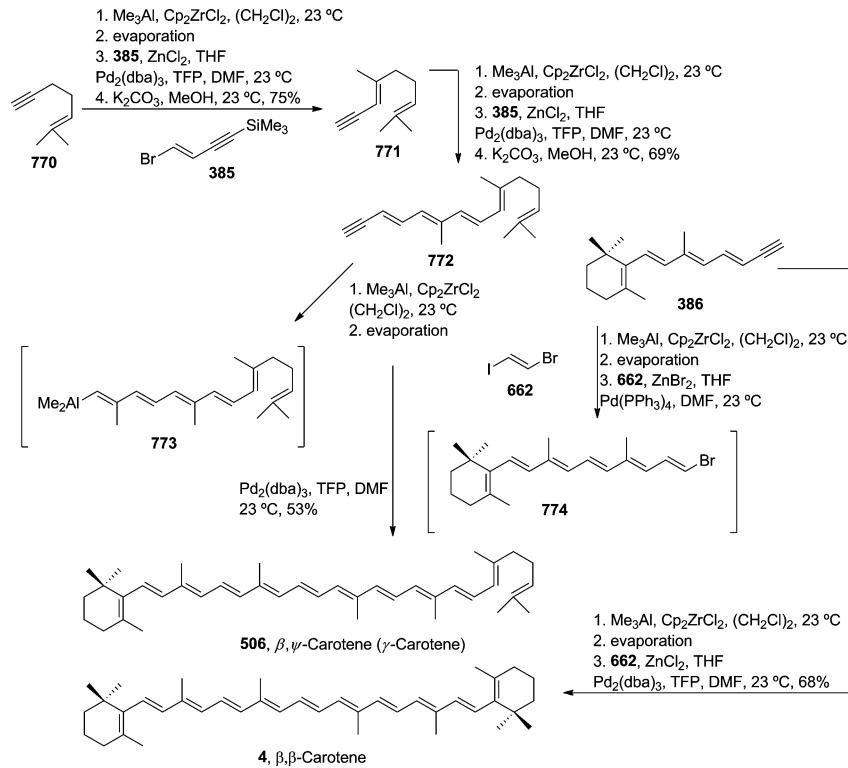
to connect the cyclic termini in a common approach to the C_2 -symmetric carotenoids cycloviolaxanthin (718) and non-symmetric capsanthin-3,6-epoxide (712) and cucurbitaxanthin A (719), a group of xanthophylls characterized by the presence of a 5-hydroxy-3,6-epoxide end group (Schemes 85 and 86).⁶⁹⁹ The selective formation of this bicyclic system was based on the regio- and stereoselective intramolecular ring-opening of the precursor epoxide catalyzed by aminium salt (p -BrC₆H₄)₃N⁺.

SbCl_6^- . This reaction was found to be general for epoxycyclohexanols with electron-withdrawing groups in unsaturated systems attached at C6 such as nitrile 740.^{699,705} For the preparation of cucurbitaxanthin A (719), the phosphonium salt 738 was protected and then reacted with dienal 705k (Scheme 81) and MeONa, and the product was treated with Dowex 50W-XH (H^+) to produce apocarotenal 739. A new Wittig reaction with 163b under the same

Scheme 88. Synthesis of Carotenoids Using Acyclic Metathesis/Dimerization

Scheme 89. Synthesis of η -Carotene (767) Using Acyclic Metathesis/Dimerization

Scheme 90. Synthesis of Symmetrical and Nonsymmetrical Carotenoids by Negishi Reaction



conditions delivered the polyene 742, which was deprotected to afford the natural carotenoid 719.⁶⁹⁹

Aldehyde 705k could be converted into the corresponding Wittig salt through the intermediacy of alcohol 743, and then treated with an apocarotenoid having the same or different terminal ring (739 and 744, respectively) to generate C₂-symmetric cycloviolaxanthin (718) or nonsymmetrical capsanthin-3,6-epoxide (712) after deprotection.⁶⁹⁹ Interestingly, the cyclopentanol terminal group of the latter compound was also obtained from the rearrangement of cyclohexene epoxides with a dienol substituent at C6 and protected (OTBS) alcohol at C3.

14.2.2. Synthesis of Carotenoids by Dimerization Reactions.

Dimerization reactions to symmetrical oligoenes offer the advantage that only a functionalized fragment is required. This was exploited by McMurry in his approach to β,β-carotene (4) by dimerization of all-trans-retinal (2) in 85% yield using low-valent titanium, in what might be considered a retro-biogenetic route (Scheme 87).⁶⁵⁹ The method is also advantageous for the preparation of ¹³C-labeled β,β-carotene from labeled all-trans-retinal.^{122b} Another application has been the synthesis of isorenieratene (504) in 96% yield⁷⁰⁶ and its 3,3'-dimethoxy analogue.^{706b}

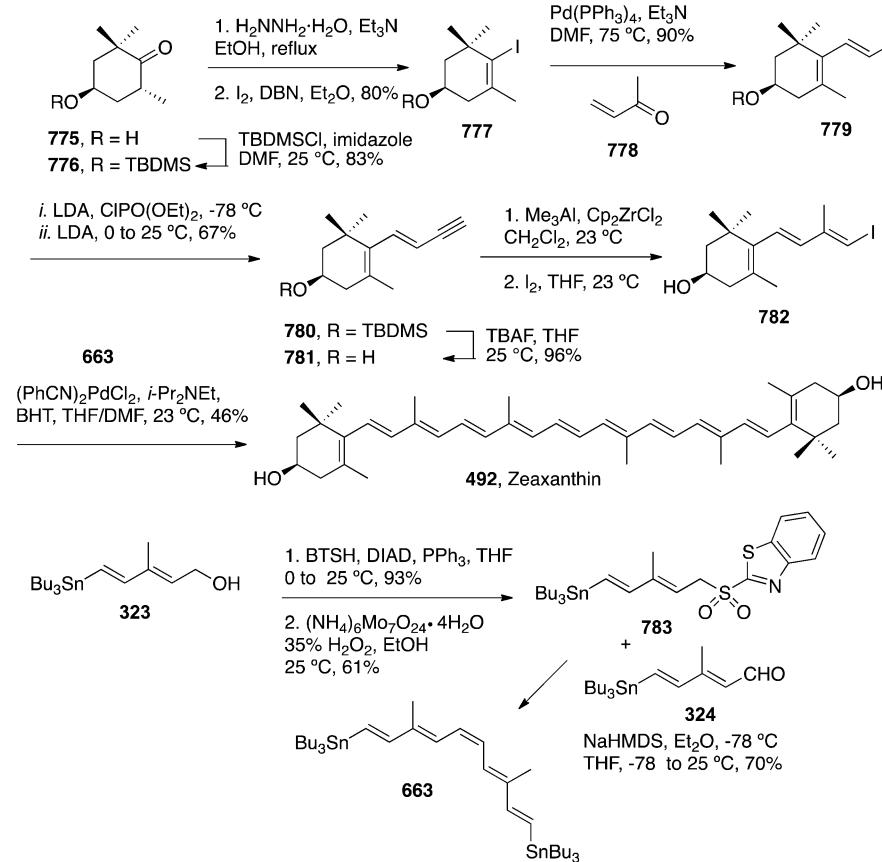
Equally powerful is a dimerization strategy based on the olefin metathesis reaction,^{301a,c,d430b,c,707} provided that the site-selectivity and the stereoselectivity can be controlled and the oligoene is stable to the reaction conditions.^{301c} Although the cross-metathesis of β,β-carotene (4) with ethylene or methyl sorbate led to complex mixtures of products in low yields,^{432b} the formation of carotenoids by homometathesis/dimerization is feasible under controlled conditions (Scheme 88).

Katsumura and co-workers established the proof-of-principle of this approach with the total synthesis of two symmetrical C₄₀ carotenoids, mimulaxanthin (709) and violaxanthin (494),^{433a}

and showed that the metathesis processes at the terminal double bond are favored over those of the tetra-, tri-, and disubstituted counterparts in the starting polyenes due to the sensitivity of the olefin metathesis initiators to steric hindrance. Concomitantly, the process was expanded to the stereoselective synthesis of other C₄₀-symmetrical carotenoids, the nonaene η-carotene (767, Scheme 89),^{433c} and four fully conjugated undecaenes (the cyclic β,β-carotene 4, the acyclic lycopene 481, and two xanthophylls, zeaxanthin 492 and *rac*-iso-zeaxanthin 706), the longest polyenes made by metathesis.^{433b} In both approaches to carotenoids by homometathesis/dimerization, the sequence requires the preparation of retinal (2) and its analogues (753–755), which are converted into the terminal alkenes (660, Scheme 70, 756–758) by Wittig reaction with 432. Optimal metathesis conditions used Grubbs' second generation catalyst (752) in dichloromethane at 50 °C for 2 h, and the carotenoids are isolated after column chromatography, virtually as single all-trans isomers, and separated in some cases from shorter fragments corresponding to minor competitive metathesis reactions.

Metathesis dimerization protocols thus constitute a novel construction strategy for these natural products, C₄₀ = C₂₁ + C₂₁ – C₂. Scheme 89 details the synthesis of η-carotene (767) from 7,8-dihydro-β-ionone (759).^{433c} Chain extension used consecutive HWE reactions, first with triethyl phosphonoacetate 256a, then with triethyl phosphonocrotonate 247. Adjustment of functional groups and final Wittig reaction led to the homodimerization precursor 766. Heating a solution of 766 in dichloromethane with catalytic amounts of Grubbs' second generation catalyst (752) produced 7,8,7',8'-tetrahydro-β,β-carotene (η-carotene) (767) in 60% yield together with small amounts of unreacted 766 (9%) and the shorter nonconjugated diene 769 (9%) and triene 768 (5%) products,

Scheme 91. Synthesis of Zeaxanthin (492) by Two-fold Stille Coupling of Homo-bis-metatalated Pentaene 663



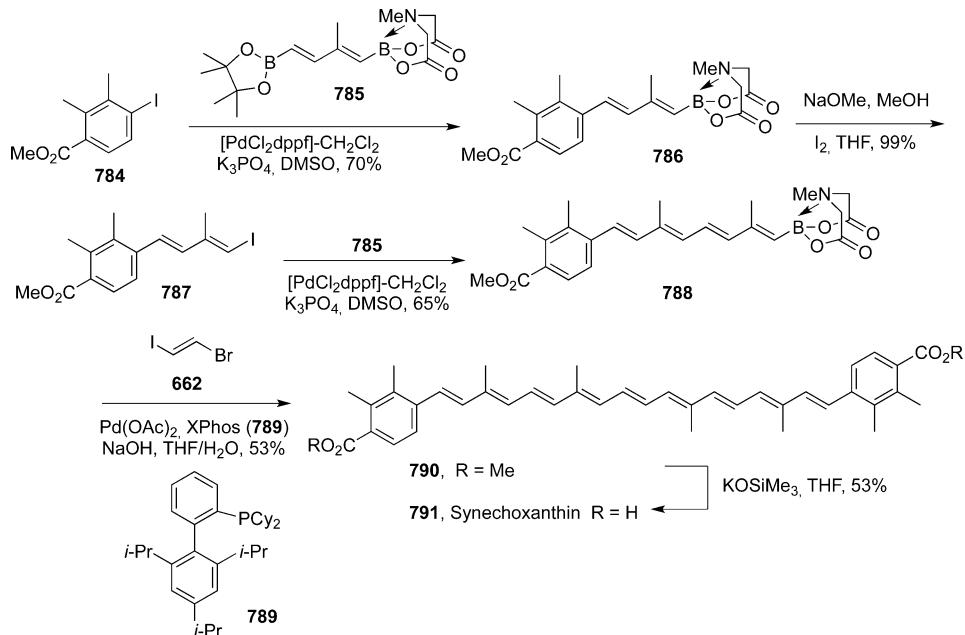
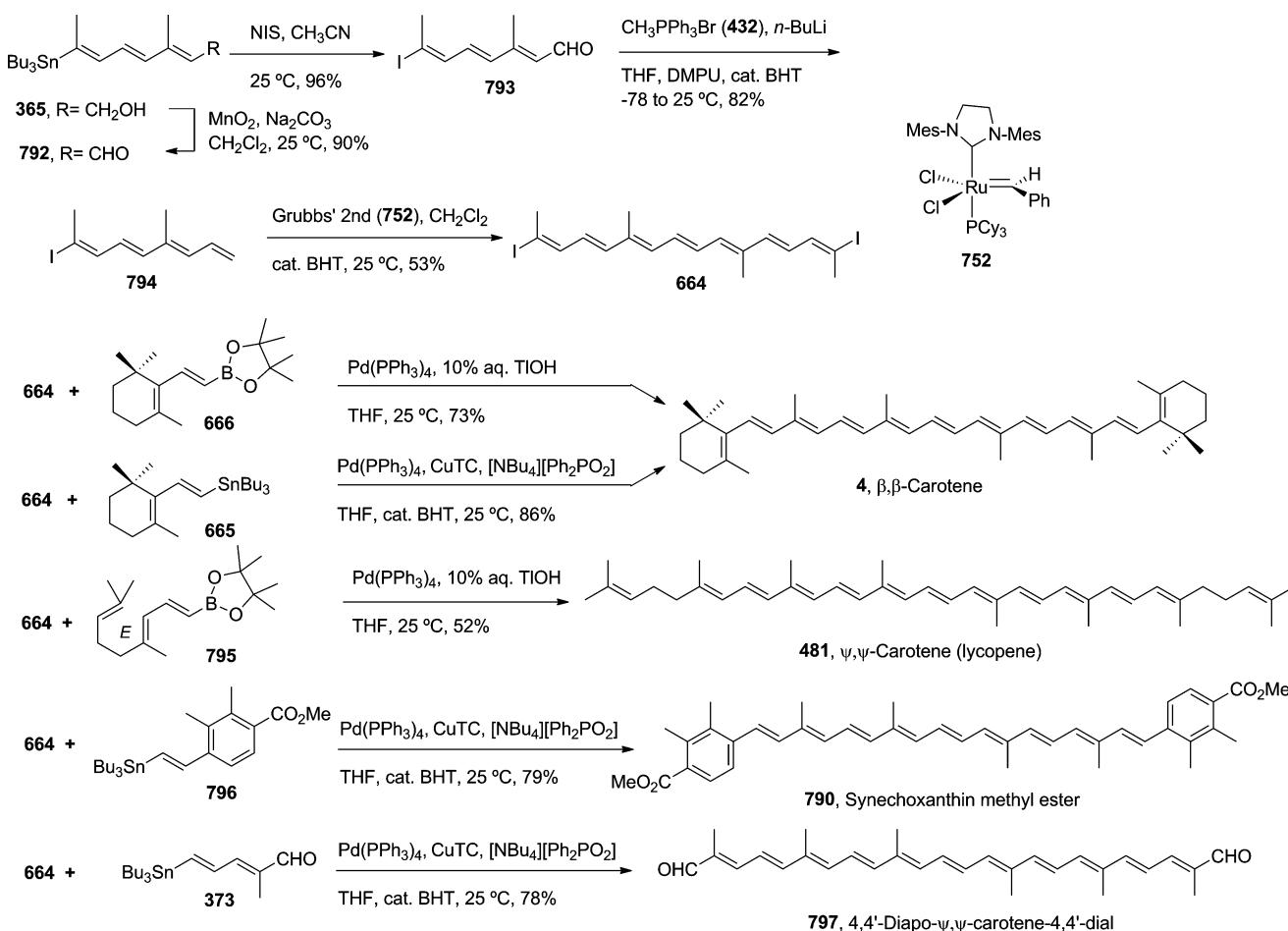
which must form through alternative metathesis of other double bonds in the starting pentaene or in product 767.

14.2.3. Synthesis of Carotenoids by Position-Selective Single Bond Forming Reactions. For polyenes with di- and trisubstituted olefins, it is crucial to shorten the number of steps in a synthetic scheme. The Pd-catalyzed alkynyl–alkenyl coupling is readily adapted to the construction of carotenoid Csp²–Csp² bonds using the appropriately matched building blocks. In addition to the components described and developed for retinoid synthesis (Figure 19), other organometallic reagents and intermediates with double functionalization are of great utility for bidirectional polyene synthesis in the case of nonsymmetrical carotenoids and also nor-carotenoids and apocarotenoids. Both homo bis-metatalated reagents with equal or unequal reactivity due to steric or electronic factors, as well as hetero bis-metatalated reagents with a latent organometal that can be unmasked at will, are present in the toolkit of functionalized fragments shown in Figure 42. Connective reagents can alternatively be functionalized with two different electrophiles, or even with an electrophile and a latent organometal, provided that the reactivity of each terminus is properly modulated. Although their application to carotenoids started in the 21st century, they nevertheless hold great promise for streamlining the synthesis of carotenoids of great complexity using iterative cross-coupling (ICC) methods in combination with stereoselective Csp²=Csp² condensation methods.

Integrated sequences comprising carbometalations/metal exchange/palladium catalyzed Negishi reactions have provided highly efficient and stereoselective routes to β,β-carotene (4) and γ-carotene (506).⁴¹⁴ The iterative and convergent synthesis of γ-carotene (506) used (E)-1-bromo-2-iodoethene (662,

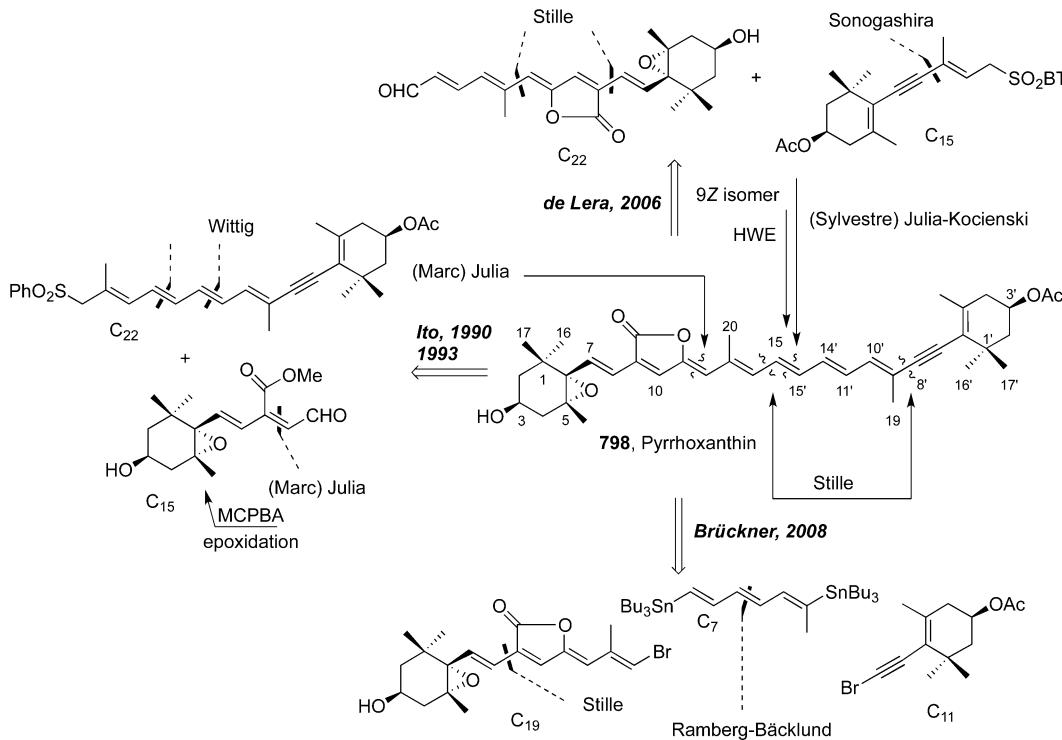
Scheme 71) as C₂ unit to connect organometals derived from alkynes 386 (Scheme 46) and 772 (Scheme 90).⁴¹⁴ These polyenyne were constructed from shorter alkynes using the Zr-catalyzed carboalumination, transmetalation to an organozinc, and palladium-catalyzed cross-coupling with either the C₄ unit (E)-1-bromo-4-trimethylsilylbut-1-en-3-yne (385, Scheme 46) or the C₂ unit 662. The methylalumination of the enyne derived from 385 provides the C5 isoprene unit of the family of natural products functionalized for the subsequent Pd-catalyzed cross-coupling. Because these protocols do not require purification of intermediates, the synthetic route to γ-carotene (506) of >99% isomeric purity involves five steps (workup and purification) from β-ionone (10) and 6-methylhept-5-en-1-yne (771) and proceeds in 32% overall isolated yield.⁴¹⁴ The 2-fold Negishi cross-coupling of 662 with the organozinc derived from tetraenyl 386 generated β,β-carotene (4) in 69% yield.

β,β-Carotene (4) and zeaxanthin (492) were made by the 2-fold Stille cross-coupling of a central C₁₂ pentaenyl-bis-stannane 663 (Scheme 71) and a C₁₄ iodide.⁶⁶⁰ The preparation of the latter in the case of zeaxanthin (492, Scheme 91) started with the Heck coupling of cyclohexenyl iodide 777 (itself obtained from enantiopure actinol 775 by protection and iodine-promoted oxidation of the hydrazone) and methyl vinyl ketone 778, and was followed by the transformation of 779 to the iodide 782 via the generation of the terminal alkyne 781. On the other hand, the pentaenyl-bis-stannane 663 was made by Julia–Kocienski condensation between stannyl BT-sulfone 783 and stannylaldehyde 324, both prepared from alcohol 323 as shown in Schemes 91 and 37, respectively. Although initially misassigned as the all-trans isomer,⁶⁶⁰ the geometry of the central double bond of

Scheme 92. Synthesis of Synechoxanthin (791) Using a Hetero-bis-metalled C₅ Reagent 785Scheme 93. Synthesis of C₂-Symmetric Carotenoids by Two-fold Cross-Coupling Reactions Using a C₁₈ Bis-iodinated Reagent 664

condensation product 663 is *cis*. The (unanticipated) Z stereoselectivity in the Julia–Kocienski reaction of unsaturated BT-sulfones and unsaturated aldehydes appears to be

general.^{369,708} Nevertheless, for synthetic purposes, this outcome was irrelevant, because the reaction conditions of the

Scheme 94. Synthetic Strategies to Pyrrhoxanthin (798), a C₃₇ Norcarotenoid

subsequent Stille cross-coupling induced the isomerization to furnish zeaxanthin (**492**) in 70% yield as all-*trans* isomer.

Bis-metallated reagent **785**, with two alkenylboron derivatives that show modulated reactivity, has been used as C₅ unit (it is also useful for terpene construction) for chain extension in the ICC approach to synecoxanthin (**791**) (Scheme 92).⁷⁰⁹ The boronic acids pyramidalized as the N-methyliminodiacetic acid (MIDA) adducts are unreactive under nonaqueous basic conditions, thus allowing one to carry out the position-selective coupling at the pinacolboronate end of **785** with iodobenzoate **784** using [PdCl₂dppf]·CH₂Cl₂ and K₃PO₄ in DMSO (Scheme 92). Boron-to-iodine exchange in the resulting MIDA boronate **786** provided iodide **787**, the substrate for a subsequent chain extension with **785** under the same conditions. Polyenyl MIDA boronate **788** was then directly employed in a 2-fold Suzuki reaction with linchpin **662**, after the reactive boronic acid was unmasked in the basic conditions, [Pd(OAc)₂, XPhos **789**, NaOH, THF/H₂O], and produced the methyl ester of synecoxanthin **790**. The carotenoid **791** was obtained by saponification of ester **790**.⁷⁰⁹

The C₁₈ heptaenylidiodide **664** (Scheme 71) is another useful conjunctive reagent for the synthesis of C₂-symmetric carotenoids (Scheme 93).⁷¹⁰ Two-directional double Suzuki or Stille reactions with appropriately functionalized termini provided a rapid entry into representative symmetric carotenoids, β,β -carotene (**4**), lycopene (**481**), synecoxanthin (**791**), and 4,4'-diapo- γ,γ -carotene-4,4'-dial (**797**). Linchpin **664** was prepared by acyclic metathesis (AM)/dimerization of tetraenylidiodide (*E,E,E*)-**794**, using Grubbs' second-generation catalyst **752** in dichloromethane and precipitated from the reaction mixture to avoid undesired isomerizations.⁷¹⁰ The use of diodoheptaenae **664** as central linchpin (the longest diiodinated polyene known) opens a novel construction tactic for these natural products, C₄₀ = C₁₁ + C₁₈ + C₁₁, according to the carotenoid synthetic terminology. This version is

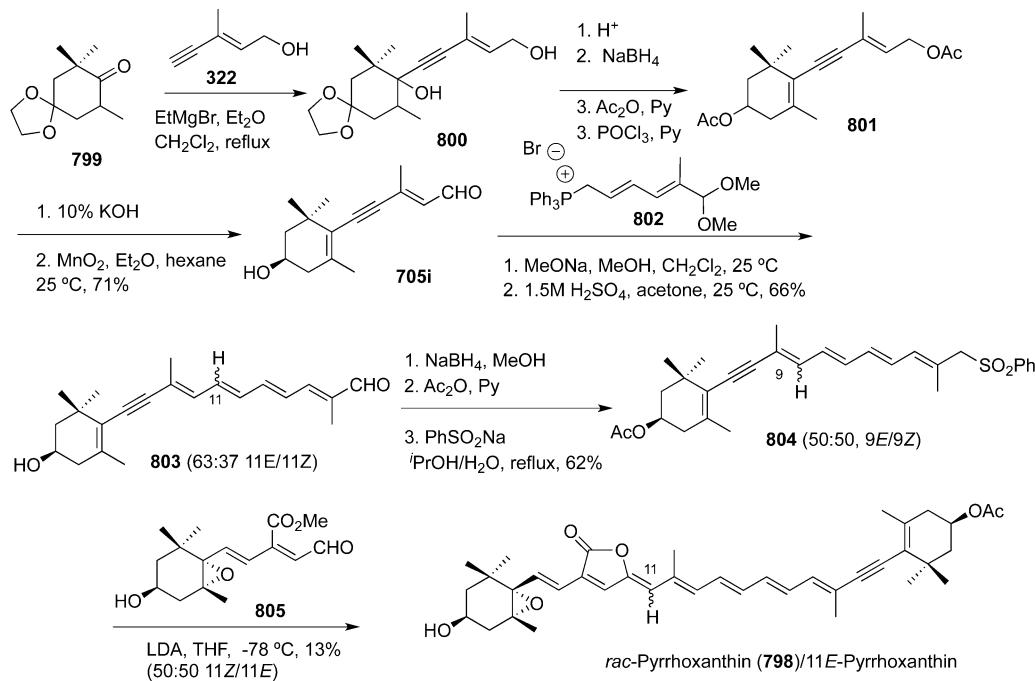
characterized by the rapid completion of the carotenoid skeleton using shorter unsaturated termini, thus circumventing the need to manipulate more unstable conjugated intermediates as in other synthetic tactics.

14.2.4. Synthesis of C₃₇ Norcarotenoids. Pyrrhoxanthin (**798**, Scheme 94) and peridinin (**513**, Figure 22) are two C₃₇ norcarotenoids isolated from planctonic dinoflagellates, with a γ -alkylidenebutenolide ring included in the polyene chain of all-*trans* geometry and two highly oxygenated cyclohex(en)yl end groups. These two xanthophylls differ by the nature of the unsaturated connection of the chain to the ring away from the common butenolide unit, an alkyne in the case of pyrrhoxanthin (**798**), and an allene in the case of peridinin (**513**). Therefore, in the latter, there are two additional stereogenic units, the cumulene axis and the vicinal tertiary carbinol stereocenter.

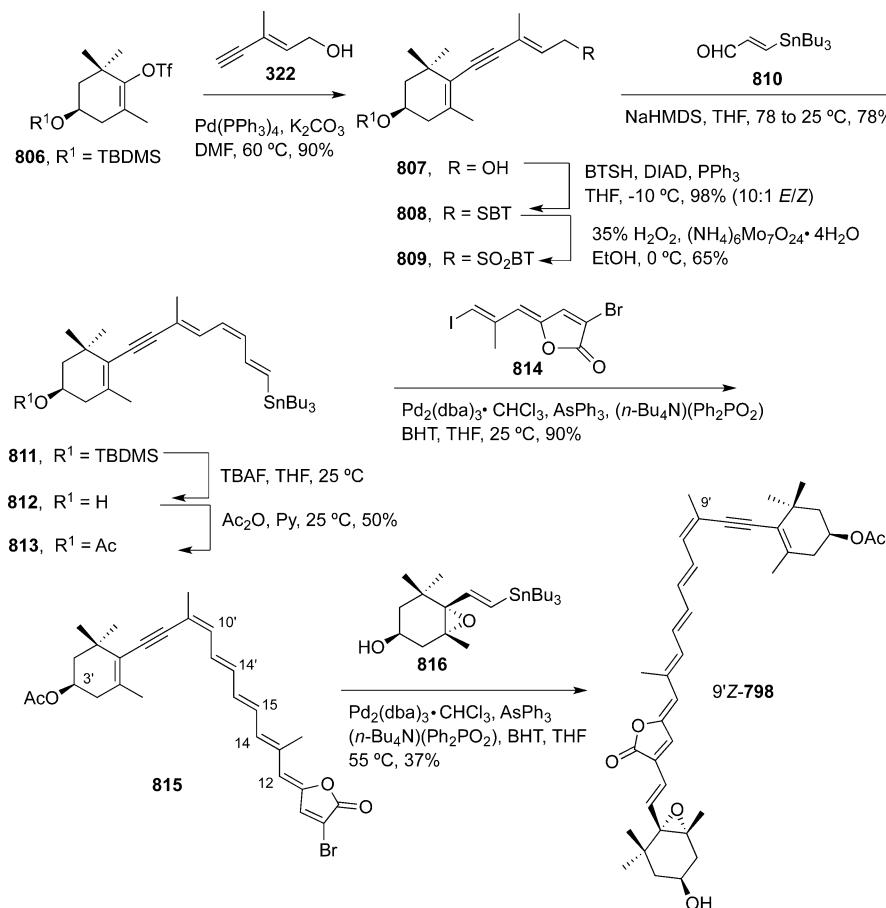
The complexity of these two oxygenated norcarotenoids has attracted the attention of synthetic chemists, and the different approaches developed over the years reflect the state-of-the-art of organic synthesis. Former approaches using condensation reactions have been more recently superseded by Pd-catalyzed Csp²–Csp² construction tactics in combination with carbonyl Csp²=Csp² olefination reactions. Moreover, using doubly functionalized unsaturated partners that can be used in tandem or in an iterative manner,^{294b} the number of steps required for the regio- and stereodefined synthesis of these structurally more complex xanthophylls is further shortened.

Pyrrhoxanthin (**798**, Scheme 94) is a member of the C7'-C8' acetylenic carotenoids, a small group of natural products known for their stereochemical lability at the enyne moiety, which shows a tendency to isomerize to the most stable 9Z isomer. Three total syntheses of pyrrhoxanthin have been described,⁷¹¹ and these are shown for comparison in Scheme 94, where the key disconnections and synthetic methods used are depicted.

Scheme 95. Synthesis of Pyrrhocanthin (798) by Late-Stage Julia Condensation



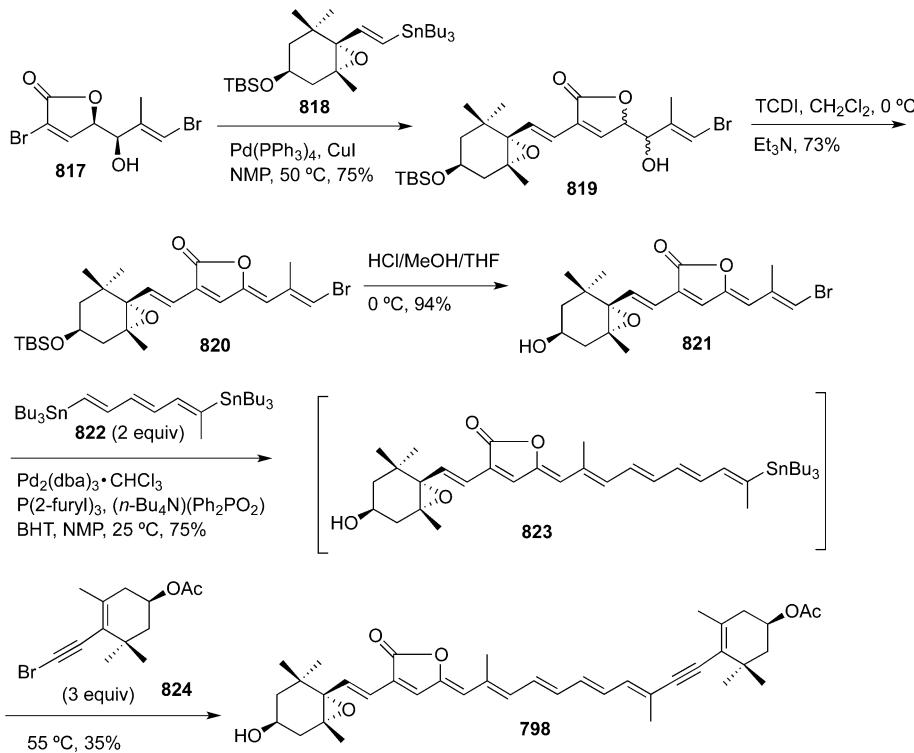
Scheme 96. Synthesis of (9Z)-Pyrrhocanthin by Consecutive Stille Reactions of a Hetero-bis-electrophile 814



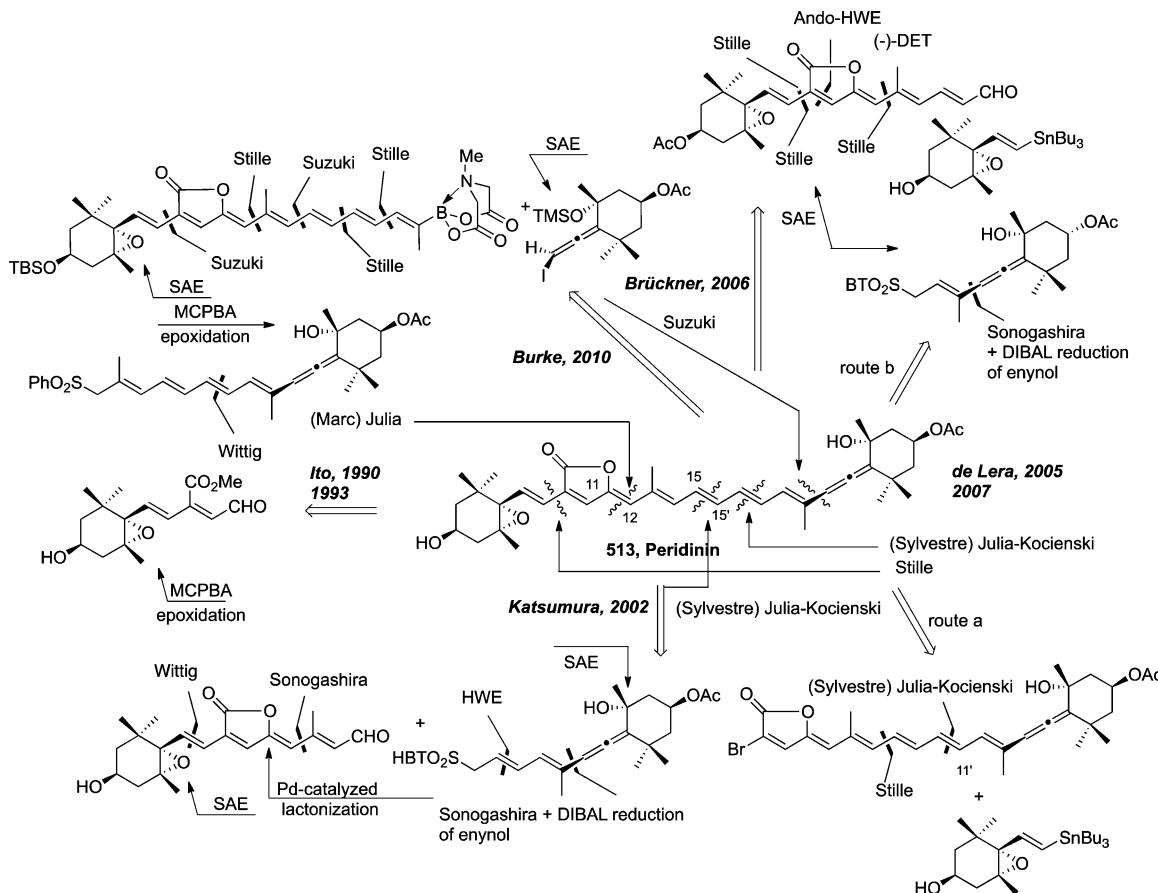
Ito and co-workers assembled the entire skeleton of pyrrhocanthin (798) as a racemate using a classical Julia reaction between aldehyde ester 805 and allyl phenyl sulfone 804 (Scheme 95). This critical step, which also constructed the

butenolide ring of *rac*-798, took place in a modest 13% yield and delivered a 50:50 mixture of the 11*E*/11*Z* γ -alkylidenebutenolide isomers.^{71a,b} Other limitations of the sequence are the

Scheme 97. Synthesis of Pyrrhocanthin (798) by Consecutive Stille Reactions of a Nonsymmetrical Homo-bis-stannane 822



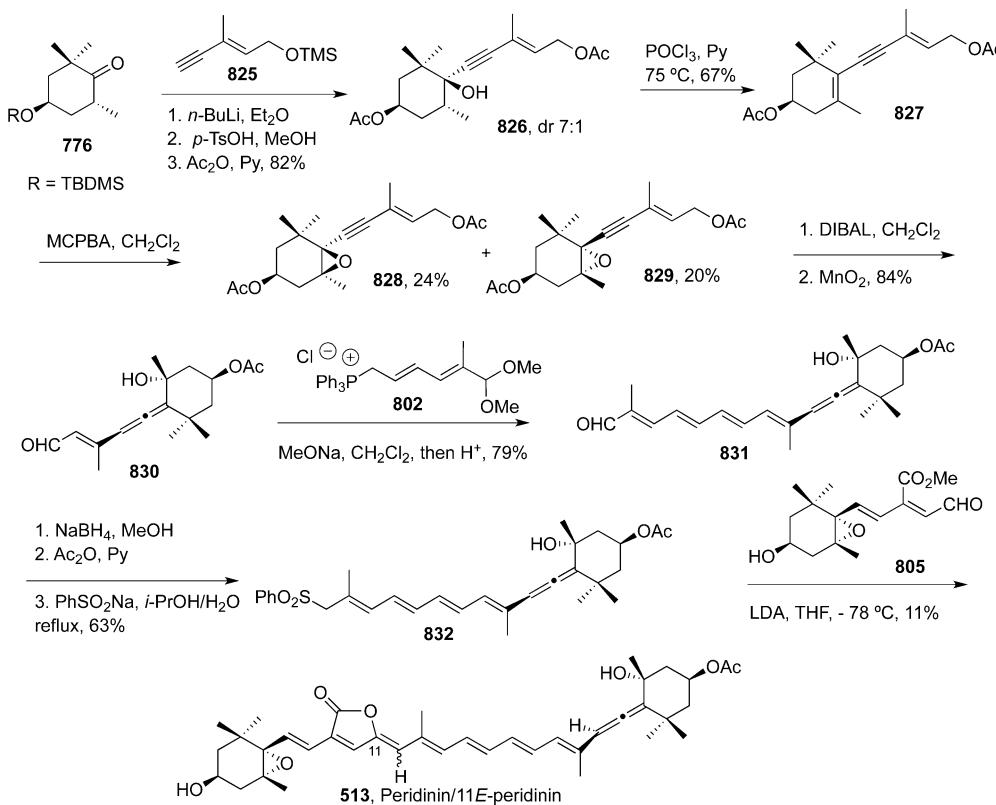
Scheme 98. Synthetic Approaches to Peridinin (513)



poor stereocontrol on the MCPBA epoxidation to arrive at C₁₅ fragment 805 and the tendency of C₂₂ alkyne 804 to isomerize.

A convergent approach to construct the complete skeleton of pyrrhocanthin by a Julia–Stille–Stille sequence afforded the 9Z

Scheme 99. Synthesis of Peridinin (513) by Late-Stage Julia Condensation



isomer of the natural product.⁷¹² Stannane 11Z-813, obtained by Julia–Kocienski reaction of 809 and 810, followed by deprotection/acetylation of the alcohol, was coupled to 2-bromo- γ -alkyldenebutenolide 814⁷¹³ in an excellent yield (90%, Scheme 96) under mild conditions using Pd₂(dba)₃·CHCl₃ and AsPh₃ as catalyst³⁹³ and (Bu₄N)(Ph₂PO₂) as Bu₃SnX scavenger.⁴¹⁰ The geometry of the reaction product was determined as 9'Z-815, which originated from a highly selective double isomerization process during the Stille coupling possibly due to palladium ligand exchange reactions involving the alkyne and neighboring olefins. The second Stille reaction of 9'Z-815 and alkanylstannane 816 required heating to 55 °C for extended periods (43 h), and provided 9'Z-798 as a single product in moderate yield (37%, Scheme 96).⁷¹²

Consecutive Stille reactions were used in the first synthesis of enantiopure natural pyrrhocanthin (798) (Scheme 97).^{711c} Bis-metallated trienyldistannane 822 was used as linchpin to connect alkenyl and alkynyl bromides consecutively, after achieving positional selectivity in the first coupling due to the lower reactivity of the stannane at the trisubstituted double bond. The butenolide moiety 820 was synthesized by the dehydration induced by 1,1'-thiocarbonyldiimidazole (TCDI) of a γ -hydroxyalkylbutenolide 819, which was obtained by another selective Stille reaction of 818 and the α -bromobutenolide unit of 817.^{711c}

The related xanthophyll peridinin (513)⁷¹⁴ has been another favorite synthetic target for the development of methodology in polyene construction (Scheme 98).^{708,711a,b,715}

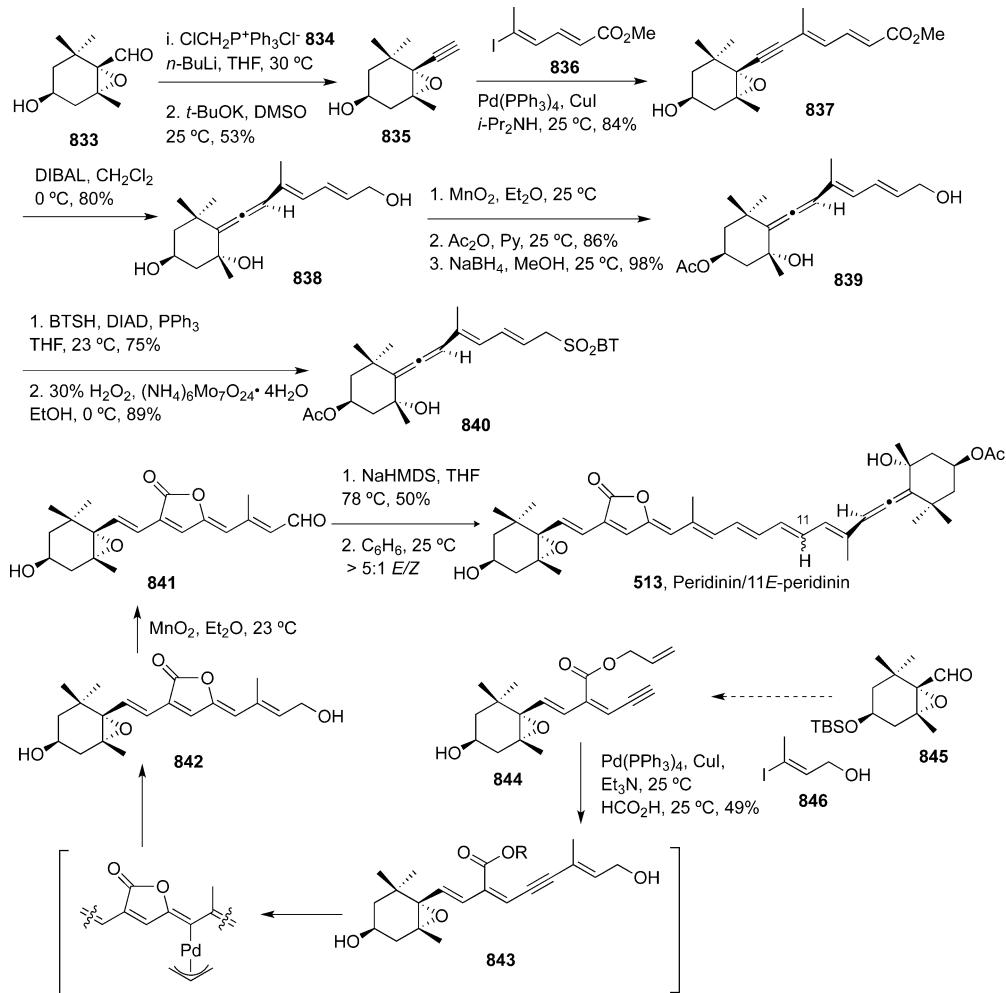
Similarly to pyrrhocanthin (798), the first synthesis of peridinin as racemate^{711a} and of enantiopure (−)-peridinin (513)^{711b} used a Julia reaction of C₁₅ and C₂₂ fragments 805 and 832 as the last step to simultaneously construct the complete C₃₇ norcarotenoid skeleton and the γ -alkyldenebutenolide

tenolide ring, as the hydroxysulfone was trapped in situ by the ester (Scheme 99).⁷¹⁶ Drawbacks of the sequence included the poor stereocontrol in some of the double-bond formation steps as well as low substrate-induced diastereoselectivity on the epoxidation of the terminal rings. The low yield and lack of stereocontrol of the Julia reaction (a mixture of the double-bond isomers at the alkylidenebutenolide was obtained) reduced considerably the efficacy of the synthesis.

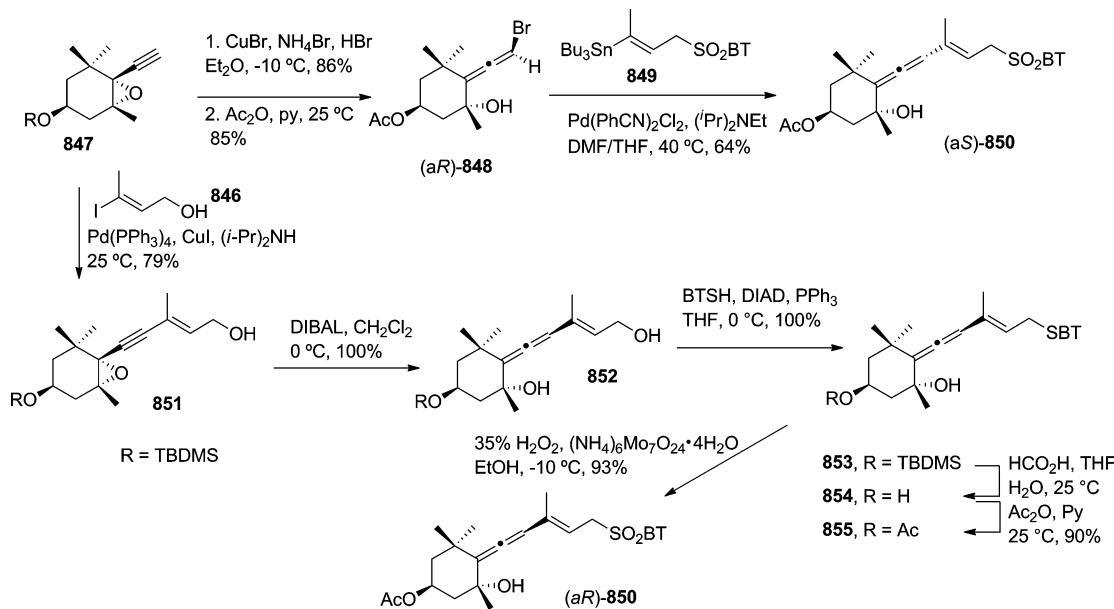
In the approach described by Katsumura and co-workers, these limitations were mostly solved, and peridinin (513) was obtained in a highly stereoselective sequence with the exception of the last Julia–Kocienski olefination of 840 and 841, which took place with 1:3 all-trans/15Z selectivity (Scheme 100). Enrichment in natural peridinin (513) (to reach a 5:1 thermodynamic equilibrium ratio) took place after stirring the mixture in benzene for 3 days at ambient temperature. In addition to the key connective step to construct the complete C₃₇ skeleton, the sequence included a remarkable Pd-catalyzed cascade, which started with the Sonogashira coupling of 844 and 846, and without isolation the intermediate 843 underwent the release of the allyl group, the 5-exo-dig anti-heterocyclization to form the butenolide ring, and protonolysis to provide 842. The configuration of the five stereocenters was derived from (−)-actinol (775, Scheme 91) and also from diastereoselective reactions including the Sharpless asymmetric epoxidation and the generation of the allene of 838 by chelation-controlled reductive ring-opening of precursor alkynylloxirane 837 with DIBAL-H.⁷⁰⁸

A different synthesis of peridinin (513) used as building blocks the central C₈- γ -alkyldenebutenolide linchpin 814 containing halogens of modulated reactivity, and functionalized C₁₁ (816) and C₁₈ (856) alkanylstannanes comprising all remaining carbons of the skeleton. By exchanging the order of

Scheme 100. Synthesis of Peridinin (513) by Late-Stage Julia–Kocienski Condensation



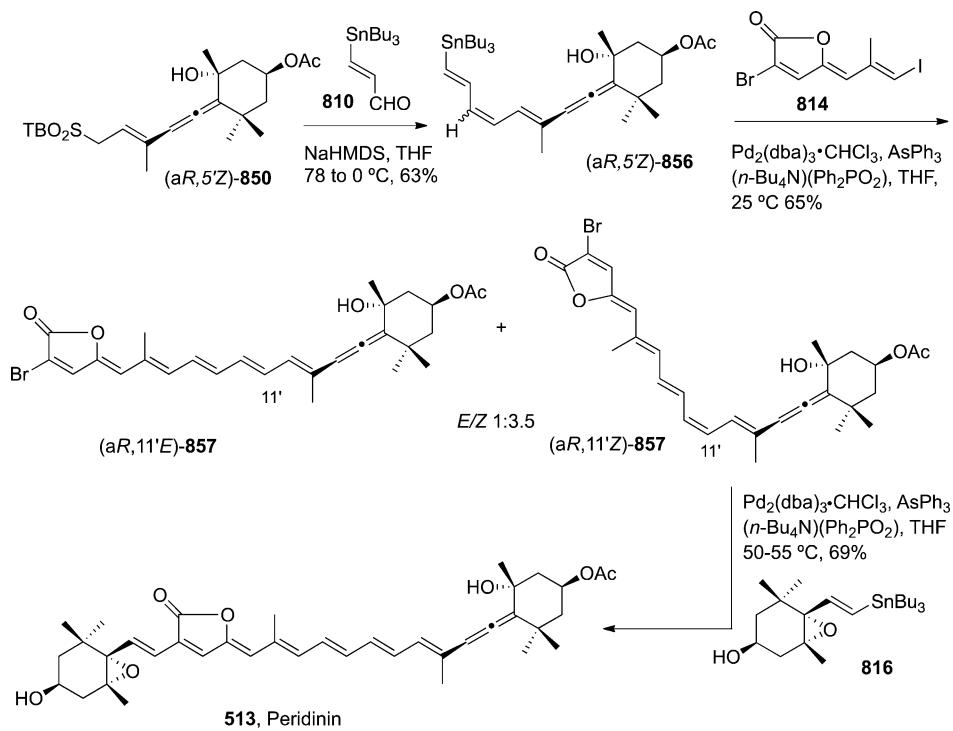
Scheme 101. Synthesis of Peridinin Building Blocks



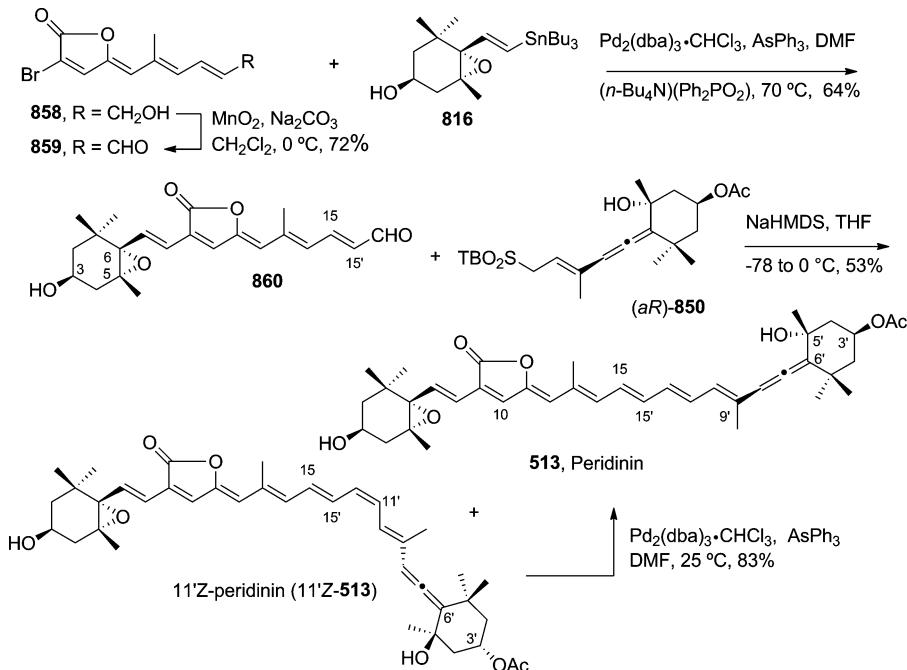
connective steps, two different construction tactics were explored (routes a and b in Scheme 98).^{715a,b}

As in the other approaches, the enantiopure cyclohexenyl fragment 850 (Scheme 101) derives from common building block (−)-actinol (775, Scheme 91).⁷¹⁷ The absolute

Scheme 102. The Stille–Julia–Stille Approach to Peridinin



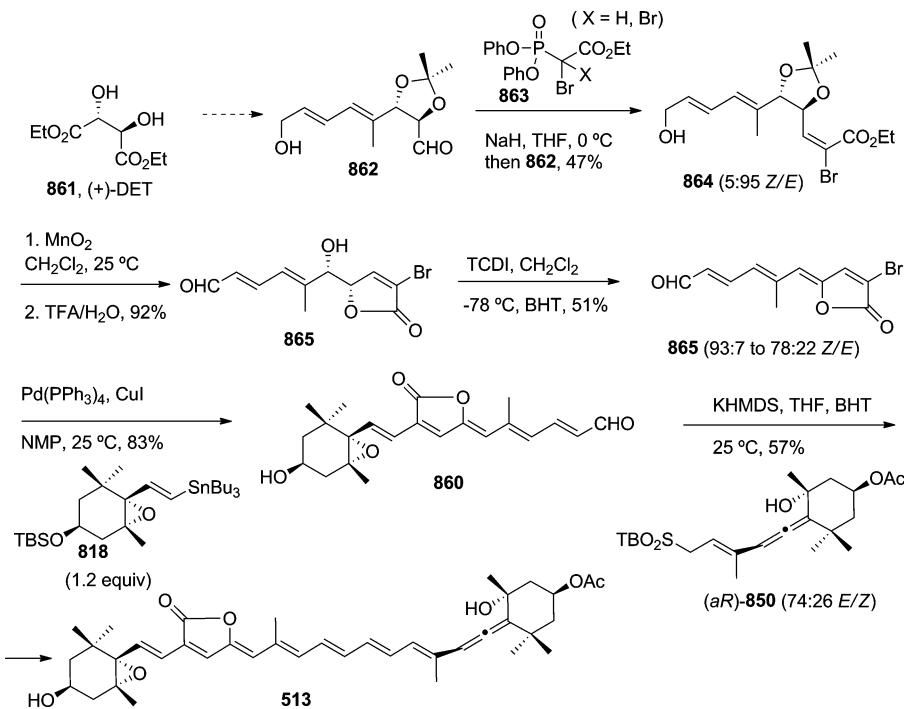
Scheme 103. The Julia–Stille–Stille Approach to Peridinin (513)



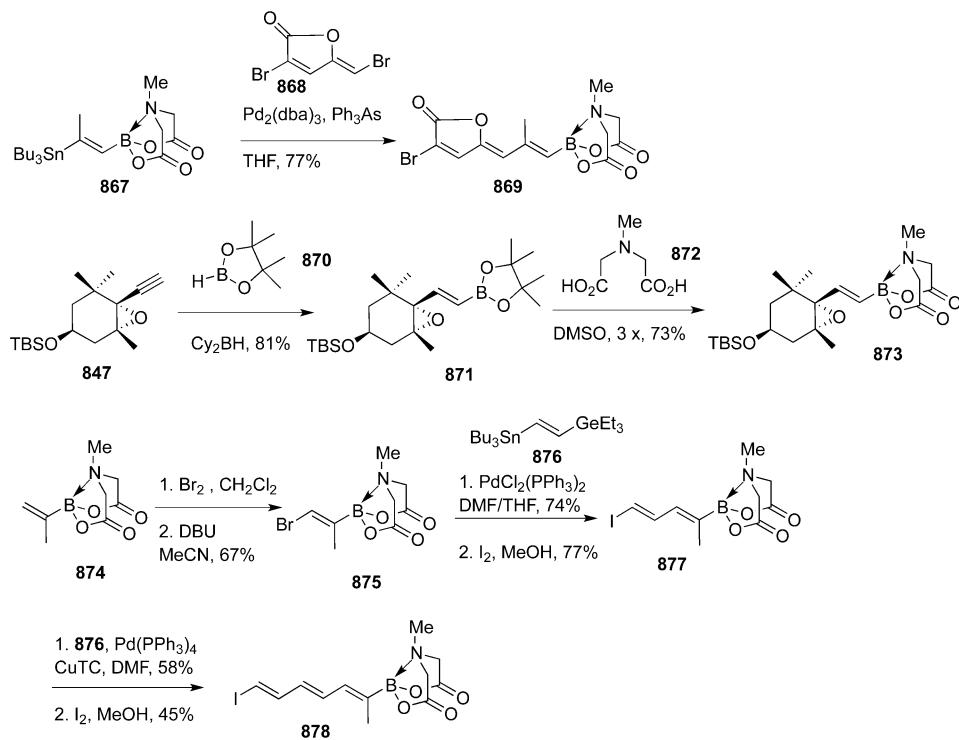
configuration of alkynylloxirane **851** was controlled by the Sharpless asymmetric epoxidation of a precursor allyl alcohol obtained from (−)-actinol (**775**), and that of (*aR*)-**850** by the S_N2'-like ring-opening of alkynylloxiranes. In contrast, the Stille coupling of bromoallene (*aR*)-**848** with alkenylstannane **849** took place with inversion of configuration to afford (*aS*)-**850**. The allene axis inversion was interpreted as a consequence of an *anti*-selective S_N2'-displacement of bromide by palladium followed by [1,3]-sigmatropic shift of propargyl- to allenyl-palladium before transmetalation and progression through the

catalytic cycle. A combined experimental and computational study on the stereochemical course of the Stille reactions of haloallenenes and stannanes led to the development of reaction conditions with stereoinversion or stereoretention outcomes.⁷¹⁸ Alternatively, the stereospecific *syn*-S_N2' reduction of the propargyl alcohol in alkynylloxirane **851** with excess DIBAL-H proceeded quantitatively (100%) to furnish **852** with complete 1,3-chirality transfer. Allenol **852** was transformed into the corresponding BT-allyl sulfide **853**, using the Mitsunobu conditions (BTSH, DIAD, PPh₃, THF, 0 °C, 100%). The

Scheme 104. The Stille–Julia Approach to Peridinin (513)



Scheme 105. Synthesis of Functionalized MIDA Boronates for the ICC Approach to Peridinin (513)



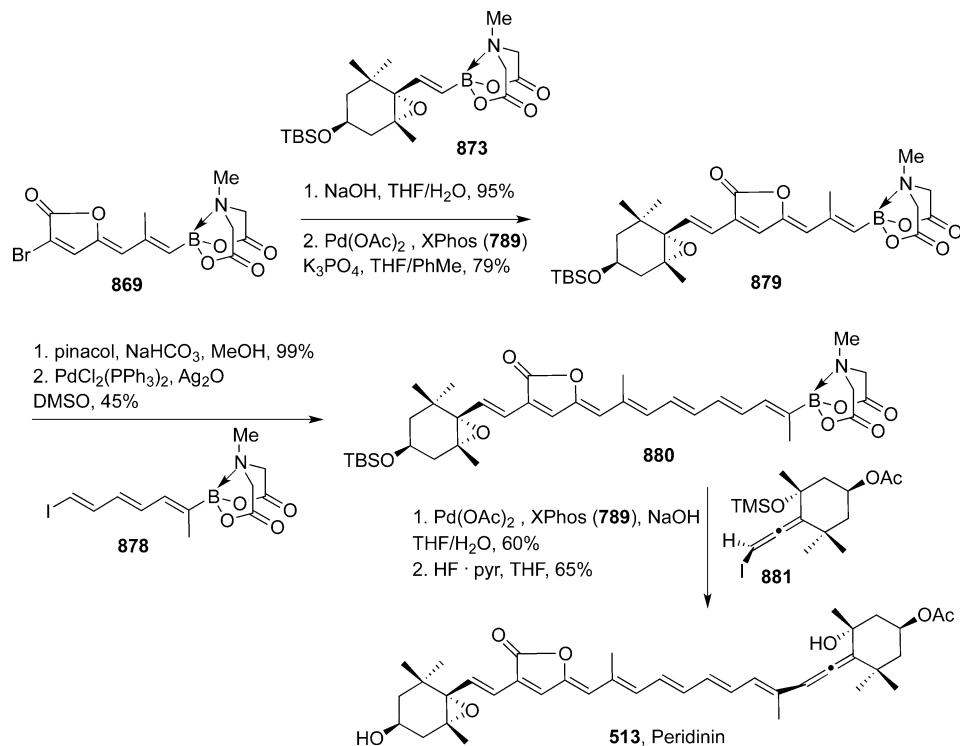
oxidation of the sulfide **855** followed the deprotection of **854** and acetylation of the alcohol **854** and provided (*aR*)-**850**.

The first C₁₈ + C₈ + C₁₁ route included a Z-selective Julia–Kocienski condensation of (*aR*)-**850** that formed the C11'=C14' olefin of **856** and two sequential Stille coupling of the dihalogenated linchpin **814** with **856** and **816** to create the C8–C9 and C14–C15 bonds.^{715a} As discussed in the case of pyrroxyanthin (Scheme 96), the reaction conditions of the last Stille reaction induced the isomerization of the polyene Z-

double bond to furnish peridinin (**513**) (Scheme 102). 6'-Epiperidinin could also be prepared by this route using BT-sulfone (*aS*)-**850**.

A closely related route, featuring a C₁₁ + C₁₁ + C₁₅ scheme (Scheme 103), disconnects the C₃₇-norcarotenoid skeleton at the C8–C9 (Stille) and the C11'=C14' (Julia) bonds after the required aldehyde **860** is constructed through another Stille coupling between **816** and **859**. An efficient Z to E

Scheme 106. The ICC Approach to Peridinin (513)



isomerization of the final carotenoid skeleton simply uses the Stille reaction conditions at ambient temperature.

A related approach was simultaneously developed, using the same late-stage connective steps, although the alkylidenebutenolide unit **865** was derived from the dehydration of intermediates obtained by synthetic modifications of (+)-tartrate **861** from the “chiral pool”.^{715c} (+)-Diethyl tartrate **861** was uneventfully converted into **862**,⁷¹⁹ which underwent Ando’s Z-selective HWE condensation²⁹⁹ with phosphonate **863** to generate the *E* isomer of the bromoester **864**. Oxidation and deprotection of the diol was followed by formation of the butyrolactone and stereoselective dehydration of **865** to **866**. Copper-accelerated Stille coupling⁴⁰⁹ of **866** with **818** provided **860**. The Julia–Kocienski condensation of **860** with the anion of BT-sulfone (*aR*)-**850** (a mixture of isomers) led to a mixture of peridinin isomers, which was converted into all-*trans*-peridinin (**513**) (Scheme 104) upon standing at ambient temperature for extended periods of time.^{715c}

The approach followed by Burke exploited the iteration of Suzuki coupling reactions of alkenyl electrophiles and boronates generated *in situ* from the tetrahedral MIDA boronates.^{715d} Because these protected organometallics are compatible with alkenylhalides (Cl, Br, I) within the same molecule, the synthesis employed C₇ and C₈ terminal ω-halo-MIDA boronates (**869**, **878**) as linchpin units. For the generation of the latter, two consecutive Stille reactions/germanohalogenation with alkenyl bromides and a (*E*)-ethylene bis-organometallic fragment (**876**) was used (Scheme 105). Bis-hetero-organometallic linchpin **867** is connected to dibromoalkylidenebutyrolactone **868** in a regioselective Stille reaction that carries over the MIBA boronate of intermediate **869**.

A first Suzuki coupling between the boronic acid obtained by hydrolysis of the MIDA boronate **873** was carried out with Pd(OAc)₂ and XPhos (789, Scheme 92) in nonaqueous

conditions to preserve the MIDA boronate present in the electrophile **869**. An additional extension of the unsaturated chain with the C₇-bis-functionalized fragment **878** produced **880**. The last Suzuki reaction of the boronic acid derived from **880** and iodoallene **881**, using XPhos (789) as Pd ligand, took place with retention of configuration of the allene axis and furnished peridinin (**513**) (Scheme 106).^{715d}

15. CONCLUSIONS AND FUTURE PERSPECTIVES

Undoubtedly, carotenoids and retinoids are the polyenes of life. Nature has fine-tuned the optical and electronic properties of carotenoids through evolution to serve specific functions, thus endowing this family of more than 800 natural products with a rich structural diversity. New structures of carotenoids are now being discovered at a faster pace than ever thanks to advances in analytical instrumentation and structural determination.⁷²⁰ Their primary role in photosynthesis is linked to the light-absorption properties of the polyene chain, but this function is additionally fine-tuned by the architectures of the supramolecular assemblies selected by nature. Xanthophylls play important functions in plants. Lutein is implicated in the structural stabilization of antenna proteins, in the capture of sunlight and the transfer of excitation energy to Chl, in the quenching of ³Chl* states, and in the photoprotection of PSII. Zeaxanthin deactivates ¹Chl* by NPQ in the antenna of PSII, and plays an antioxidant role in the lipid phase of the thylakoid membrane, particularly under high light stress conditions. The central role of zeaxanthin and lutein in the photoprotection of photosynthetic organisms is paralleled in the human eye, where they might contribute to reduce the risk of developing AMD and cataracts. It is the ability of the polyene chain to absorb light and extinguish reactive species (ROS, radicals) that not only protects the photosynthetic devices but also the macula lutea and presumably other tissues in animals, thus accounting for the health benefits of the carotenoids. Moreover, the

cleavage of carotenoids by carotenoid cleaving enzymes (CCes) generates a rich diversity of metabolites, the apocarotenoids, that are used for a rich variety of natural functions that continue to fascinate scientists across all disciplines. The most important of these apocarotenoids are vitamin A and derivatives.

The apparently deceptively simple polyene structure of vitamin A (**1**), a hydrocarbon with a functional group, encodes an extraordinary potential for diversification of this family of compounds to trigger a plethora of biological activities. This diversity has been instrumental during evolution to set up entirely distinct body functions, such as embryonic development and organ homeostasis in which all-*trans* retinoic acid (**3**) plays an essential function and vision for which 11-*cis*-retinal (**5**) is the key metabolite. These diverse functions of vitamin A metabolites in the body, still far from completely understood, explain the enormous pharmacological importance of synthetic derivatives that aim to specifically address particular pathologies, while avoiding toxic or teratogenic affects that are an inherent consequence of the importance and complexity of this system.

Aberrations within the system have told us key messages and even led to the cure of the associated pathologies. Historically, the first aberrations were recognized from deficiency of this vitamin (from the mistaken notion that they corresponded to “vital amine”) due to either malnutrition or experimental removal from the diet of animals. These data revealed implication of vitamin A in vision,¹⁶¹ reproduction and embryonic development,⁷²¹ immune response,⁷²² and neural functions.²⁹⁰ Some, but not all, of these effects were caused by deficiency of retinoic acid at certain stages of development and in particular organs,⁷²³ and some of these vitamin A deficiency syndrome (VAD) features could be linked to specific retinoic acid receptors by genetic engineering of receptor-deficient/mutant mice.⁷²⁴ A highly specific aberration, a somatic mutation originating from a chromosomal translocation, revealed the impact of all-*trans* retinoic acid and RAR α in a pathology blocking the maturation of the myeloid lineage at a particular stage and paved the way to the cure of one of the deadliest leukemias.²⁸ However, while these two key observations emphasize the importance of the system, we are still far from understanding the underlying complexity, and its medico-pharmaceutical potential is by far not exploited, as we learn from recent studies. Below we discuss some of the areas where we see the need for future studies and potential for drug development.

15.1. Chemistry and Biotechnology

From the pharmacological perspective, new metabolites, designed to exert precise biological functions, can be generated by enzyme-assisted modifications of the double bonds and/or the allyl positions of the polyene chain. In addition, the prosterogenic nature of the tri- and tetrasubstituted double bonds of the polyene chain provides the possibility of generating additional enantiopure retinoid metabolites. In this regard, advances in vitamin A enzymology, transport, and metabolism reveal important insights in the role of this small family of ligands in lipid homeostasis and other pathways that impact on health and disease.

Moreover, the formation of Schiff bases of a retinal aldehyde with appropriate receptors has been chosen by nature as biological tools to exploit the conjugated makeup of the polyene for sensing environmental sources of light. Rhodopsins

play in nature apparently unrelated functions, but some general principles are emerging. Regardless of the microbial or animal origin, Rhs function as light-driven transporters and as light sensors. In the first case, halophilic bacteria (bR, HR, xanthorhodopsin), marine bacteria (proteorhodopsin), and fungi (*Leptosphaeria* Rh) use the complexes to control an electrochemical membrane potential to generate ATP. The light-sensing activity of mammalian rod Rh, the color visual pigments in vertebrate and invertebrates, and SR-I and SR-II in halobacteria convey signals from protein–protein interactions to integral membrane transducer proteins (transducin in Rh, Htr in SR), which triggers the cytoplasmatic phosphorylation pathway (Htr/SR) or GTP/GDP exchange (Rh/transducin). New Rhs (such as Middle Rhodopsin) that share features of both functions (bR and SRII) are important for evolution studies of this family of retinal-based proteins.

In addition to the exciting findings on the evolution of the opsin family of proteins, Rhs have potential for biotechnological applications. Interestingly, ChR2 has been functionally expressed in mammalian neurons where it is able to drive light-induced neuronal depolarization, and is used in optogenetics as a tool to investigate the cellular excitation/inhibition balance in information processing.^{150,725} In this growing field of optogenetics, it was also found that archaerhodopsin-3 (Arch-3) from *Halorubrum sodomense*, a light-driven outward proton pump, when genetically expressed in neurons, enables them to be powerfully, transiently, and repeatedly silenced in response to pulses of light.⁷²⁶

The same polyene nature of retinoids and their biogenetic precursors, the carotenoids, has been a synthetic challenge for decades. Undoubtedly, their structure has been an inspiration for the development of methodologies for double-bond constructions, and the industrial synthesis of retinoids has benefited from the novel methods. A new generation of synthetic methods, conceptually based on the alternative construction of single bonds between functionalized unsaturated centers,³⁰⁰ has emerged more recently, and its application to retinoid synthesis has streamlined the preparation of many members of this family of polyenes.

As a result of the interplay of molecular biology, structural biology, and organic synthesis, a series of synthetic retinoids and rexinoids, ligands for the RXR and RAR with well-defined functions (agonists, antagonists, partial agonists, inverse agonists, which modulate the structure of specific receptor surfaces that serve to communicate with other regulators), is emerging. Indeed, an enormous variety of receptor processes can be collectively or separately addressed by modifying the ligand structure to produce so-called SNuRMs (specific NR modulators^{23b}), and we have begun to understand and pharmaceutically exploit the corresponding mechanisms, as in the case of LG101506 (**96**) and compound **95**, selective RXR/PPAR γ and RXR/LXR heterodimer selective modulators, respectively.

The existence of a natural “bona fide” RXR ligand remains an unsolved issue. While the existence and (autonomous) signaling function of 9-*cis*-retinoic acid (**7**) in vivo lacks confirmation, docosahexaenoic acid (DHA), a fatty acid abundant in mammalian brain cells where it is found primarily associated with membrane phospholipids, has been characterized as a natural RXR ligand. It is essential for brain maturation, and DHA deficiency leads to impaired brain functions in rodents and humans. Notably, DHA accumulates in the retina, a tissue that develops abnormally in RXR α

knockout mice. Indeed, deficiency of DHA in rats and humans results in several abnormalities that are similar to those seen in RXR α -deficient mice.⁷²⁷ Whether other unknown retinoid metabolites bind to and activate RXR at physiological concentrations remains to be demonstrated.

15.2. The Need for a System Biology Analysis of Retinoic Acid Receptor Signaling

The initial events following binding of a ligand to its cognate receptor, the induced allosteric conformational changes, the subsequent alteration of the surfaces to which coactivators or corepressors can bind, and the corresponding establishment or dissociation of epigenetically active coactivator and corepressor complexes and recruitment of transcription and chromatin remodelling complexes, are at least in part well understood. However, the analysis of the corresponding dynamic regulation of gene networks that are the ultimate cellular readouts of ligand action is still in its infancy,⁷²⁸ and predicting the (epigenetic) key factors involved in the regulation of the circuits that govern proliferation, survival, and cell death is a promising holistic approach to identify novel drug targets. Such approaches are now feasible due the technological advances in high throughput sequencing, and the existence of powerful OMICS technologies, bioinformatics tools, and rapidly developing systems biology.

15.3. Cancer Therapy and Prevention⁷²⁹

The cure of acute promyelocytic leukemia (APL; discussed above) has been one of the (rare) success stories in cancer therapy. While this is often considered a rather isolated case due to the specific chromosomal translocation that generates the disease, there is increasing evidence that the cell differentiation and apoptogenic action of retinoids is blocked also in non-APL leukemias, and this block can be released by adequate combinatorial treatment, for example, with signaling compounds or epigenetic drugs (epi-drugs). There is significant hope for novel types of combinatorial treatments, which sensitize non-APL acute myeloid leukemias and solid tumors to the differentiation and/or apoptosis-inducing actions of retinoids and rexinoids.^{280c,281b,282,283}

Studies mapping the estrogen and retinoic acid receptor binding sites ("cistromes") in the genome of breast cancer cells revealed that there is a significant crosstalk between these two receptors systems.⁷³⁰ For both of these receptors, a large number of ligands have been developed, inviting studies to explore their combined use for control of cell proliferation and/or induction of apoptosis.

Subsequent to disappointing results in some chemoprevention studies, recent studies point to novel avenues in this direction by combining retinoids with nongenotoxic apoptogenic agents.²⁸⁵ Moreover, by considering the divergent genetic makeup of patients, drug responders may be identified and treated by personalized cancer prevention strategies.²⁸⁹

15.4. Metabolic Syndrome and Alzheimer's Disease

In addition to their applications in cancer,^{280a,b} rexinoids could be valuable in the treatment of diabetes and obesity (two major components of the Metabolic Syndrome), as well as atherosclerosis, other cardiovascular indications, and inflammatory diseases,^{280b} via signaling pathways that depend, among other factors, on the modulation of the heterodimers with PPARs⁷³¹ and LXRs.^{85,732}

An exciting novel activity is the bexarotene-induced ApoE-mediated clearance of β -amyloid (Ab) from the brain in a

mouse model of Alzheimer's disease,²⁷⁹ which may be revisited in the context of the above-mentioned brain functions of RXR α and rexinoids.

Taken together, there are exciting novel avenues to unravel the complex biology of the retinoid and rexinoid functions in the body and to exploit the multiple activities of (chemically engineered) retinoic acids for the therapy of a large variety of diseases. Toward this goal, it will be important to understand the action of the various members of the RAR, RXR, and RXR partner families from a systems biology perspective and to combine this knowledge with patients stratification strategies that include global (epi)genetic profiling to identify responders and develop the corresponding companion diagnostics.

15.5. Carotenoids in LHCs

The crystal structures of LHCs^{458,467a,468} are providing useful information on the role of carotenoids as natural antennas for light capture and energy transfer to acceptor chlorophylls. Spectroscopic studies of the isolated carotenoids will be necessary to correlate their solution properties with those optimized by the protein environments in the natural systems, and thus advance in the design of nanoscale antenna devices for artificial photosynthesis and solar energy conversion.

15.6. Carotenoids as Antioxidants

The proven capacity of carotenoids to affect oxidative processes in biological systems and scavenge radicals has been considered to provide health benefits. However, recent data obtained from microscope-based time-resolved spectroscopy to measure single oxygen concentration in mammalian cells have strikingly shown that β,β -carotene does not efficiently deactivate $^1\text{O}_2$ produced by a photosensitized process.⁷³³ The failure of β,β -carotene to alter the lifetime of intracellular O_2 , as compared to its efficient role in the solution-phase deactivation of $^1\text{O}_2$, was ascribed to the decrease in the rate of collision for productive quenching interaction. In plants the deactivating carotenoids are placed adjacent to the $^1\text{O}_2$ -producing chromophores, avoiding the diffusion problems of mammalian cells. Thus, the role of β,β -carotene as an antioxidant *in vivo* requires reevaluation.⁷³³

Astaxanthin is another powerful lipophilic antioxidant and a potential therapeutic agent for treating cardiovascular disease and prostate cancer,⁷³⁴ but its bioavailability is limited due to poor oral absorption. In recent human clinical trials, it has been found that astaxanthin modestly reduced triglyceride levels, but showed anticancer, antidiabetic, and anti-inflammatory effects. Oral astaxanthin pro-drug CDX-085 (the structure of which has not been disclosed) distributes among lipoproteins and was found to lower total cholesterol and aortic arch atherosclerosis in $\text{LDLR}^{-/-}$ mice and triglyceride levels in $\text{ApoE}^{-/-}$ mice, and is therefore a promising candidate for further evaluation in human studies.⁷³⁵

Preclinical studies involving other carotenoids, such as the end-aromatic carotenoids 3,3'-dihydroxyisoreineratene and isorenieratene, are warranted as these polyenes might be multifunctional photoprotection agents for the prevention of skin cancer. In cellular studies, these compounds prevented UV-induced DNA damage in cultured human skin fibroblasts, reduced UV-induced cyclobutane pyrimidine dimers, and UV- and oxidatively induced DNA strand breaks and lipid oxidation (the latter effect was not observed with lutein).⁷³⁶

15.7. Signaling by Apocarotenoids

More than 100 naturally occurring members of the apocarotenoid subgroup are known. In addition to the

multifunctional biological activities described above, some of these compounds show greater potency in the inhibition of cancer growth than intact carotenoids, and modulate the activity of various transcription systems, including nuclear receptors RAR, RXR, PPAR, and ER and other proteins involved in cancer, such as NF κ B.⁷³⁷ The recent discovery that the phytohormone abscisic acid (ABA) is involved in inflammation has shown the evolutionary adaptation of a hormone to play different signaling functions (stress signal in plants, cyclic ADP-ribose-dependent signaling in human granulocytes) from plants to humans.

15.8. Chemopreventive Effects of Carotenoids

Recently published epidemiological studies concur that there is no association between the consumption of fruits and vegetables and the incidence of common cancers or coronary heart diseases. Similarly, RCTs indicate the lack of correlation between carotenoid supplementation and prevention of cancer. The promising small-scale studies on lutein/zeaxanthin supplementation and AMD progression in the elderly will be confronted with the results of an ongoing RCT study (AREDS2) on the risk of developing AMD in subjects taking supplements containing 10 mg of lutein and 2 mg of zeaxanthin a day. The preventive and beneficial effects, the carcinogenicity, and other toxic effects of these compounds continue to be investigated with better animal models and better biomarkers, more intervention studies, and investigations of food composition, to develop a better understanding of the metabolism of carotenoids and mechanisms of carcinogenesis.

15.9. Carotenoids and Nutrition

A recent survey has concluded that the β,β -carotene mean intake of 3.9 mg/day contributes approximately to 65% of the total vitamin A supply (mean daily RAE of 1083 ± 175) in industrialized countries.⁷³⁸ Understanding the control of carotenoid biosynthesis will impact on crop nutrition. The genetical engineering of Golden Rice⁷³⁹ that is rich in carotene has generated a promising effective source of vitamin A for children in developing countries.⁷⁴⁰ In a clinical trial, β,β -carotene in Golden Rice was found to be as effective as pure β,β -carotene in oil and better than that in spinach at providing vitamin A to children.^{740c,e} Although the validity of the findings for human nutrition in populations at risk of vitamin A deficiency has been questioned,^{740d} such developments will help eradicate the public health threat of vitamin A deficiency.

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Notes

The authors declare no competing financial interest.

Biographies



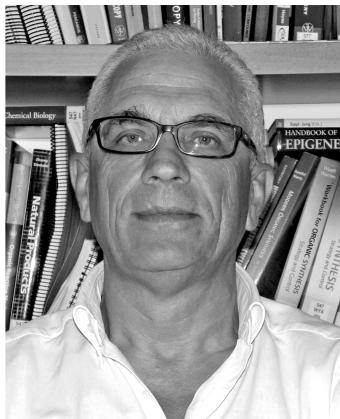
Prof. Rosana Álvarez received a Ph.D. in 1997 from the University of Vigo. After a postdoctoral stay at the University of Paris-Sud (Prof. Jean D'Angelo), she started her career in 2001 as "Ramón-Cajal Senior Researcher" centered on the stereocontrolled synthesis of retinoids and carotenoids based on transition metal-catalyzed reactions. She was promoted to Full Professor at the University of Vigo (2011) and continues her research in polyene synthesis, in particular of retinoids and carotenoids, and more recently in the experimental and theoretical study of transition metal catalyzed, including bimetallic catalysis, and organo-catalyzed reactions to establish their mechanism and to design new synthetic methodology.



Dr. Belén Vaz obtained her Ph.D. in Chemistry from the University of Vigo in 2004 for her work on the stereocontrolled total synthesis of highly functionalized carotenoids via palladium-catalyzed cross-coupling reactions. In 2005, she started a postdoctoral stay in the group of Prof. Luc Brusveld in the Max-Planck Institute of Molecular Physiology in Dortmund (Germany). During this period, she was engaged in a drug discovery program oriented to the modulation of protein–protein interactions and targeting nuclear receptors. In 2007 she returned to the University of Vigo as a Research Associate. Her current research interests focus on the synthesis of complex polyenes and oligopyrroles assisted by transition metal catalysts, the structural determination of natural marine pigments (chlorophylls and carotenoids), and the incorporation of small molecules to nanostructured materials for the design of SERS-based sensors as well as the development of plasmonic nanoreactors.



Dr. Hinrich Gronemeyer works at the GBMC in Strasbourg-Illkirch. Trained as chemist, he did his Ph.D. in Biochemistry at the RUB Bochum, Germany, and joined as postdoc the team of Pierre Chambon (Strasbourg, France), where he became head of the Cell Biology and Signal Transduction and Cancer Biology departments in the newly founded IGBMC. He is Research Director (Class "Exceptional") of the French National Institute of Health and Medical Research (INSERM) at the Department of Functional Genomics and Cancer of the IGBMC. He had extensive collaborations with the pharmaceutical industry. He has a long-standing experience in the field of nuclear receptors, with a particular focus on the structure-function characterization, and modulation of retinoid receptors in health and disease. Presently, his team studies nuclear receptor action from a systems biology perspective to reveal the temporal regulation of gene networks and protein diversification by epigenetic actors.



Prof. Angel R. de Lera received a Ph.D. in 1983 from the University of Santiago de Compostela. After a postdoctoral stay at the University of California, Riverside (Prof. W. H. Okamura), he started his career in 1987 as Assistant Professor at Santiago de Compostela with a research program centered on the design, stereocontrolled synthesis, and biological evaluation of retinoids, and the study of their properties, reactivity, and interactions with a variety of receptors (rhodopsin, bacteriorhodopsin, the retinal dehydrogenases, and retinoid receptors). He was promoted to Full Professor at the University of Vigo (1998) and continues his research in polyene synthesis, reactivity, and biology, in particular of retinoids and carotenoids, and more recently in the design and synthesis of modulators of the chromatin-remodeling enzymes (epi-enzymes) inspired by the structure of natural products. He has been a Visiting Scholar at the University of Washington in Seattle (1996) and a Visiting Professor at the Albert-Ludwig Universität in Freiburg (2000).

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ABBREVIATIONS

A2-DHP-PE	N-retinylidene-N-retinyl-dihydropyridine-L- α -phosphatidylethanolamine
A2-E	N-retinylidene-N-retinylethanolamine
A2PE	dipalmitoyl-L- α -phosphatidylethanolamine
Ab	β -amyloid
ABA	abscisic acid
ABC	ATP-binding cassette transporters
ACR	acylic retinoid
AD-mix	asymmetric dihydroxylation mixture
AKR	aldo-keto reductase
AIBN	azo-bis-isobutyronitrile
ALDH	aldehyde dehydrogenase
AM	acyclic metathesis
AMD	age-related macular degeneration
AML	acute myelogenous leukemia
AMP	adenosyl monophosphate
APC	adenomatous polyposis coli
APL	acute promyelocytic leukemia
Apo-E	apolipoprotein-E
AR	anhydroretinol
aR2	archaerhodopsin-2
Arch-3	archaerhodopsin-3
AREDS	age-related eye disease studies
ATBC	α -tocopherol β -carotene
ATR	all-trans-retinal dimer
ATR-PE	all-trans-retinal dimer-L- α -phosphatidylethanolamine
(U)B3LP	(unrestricted) Becke-3-Lee-Yang-Parr
BChl	bacteriochlorophyll
BCMO1	β,β -carotene-15,15'-monoxygenase 1
BCDO2	β,β -carotene-9',10'-dioxygenase 2
BHT	butylated hydroxytoluene
BL	Burkitt's lymphoma
bR	bacteriorhodopsin

BT	benzothiazol-2-yl	HO-1	heme oxygenase-1
CARET	β -carotene retinol efficacy trial	4-HPR	all- <i>trans</i> -retinoic acid <i>p</i> -hydroxyanilide
CASPT2//CASSCF/AMBER	complete active space perturbation theory//complete active space self-consistent field/assisted model building with energy refinement	HR	halorhodopsin
CBS	Corey–Bakshi–Shibata	14-HRR	14-hydroxy-4,14- <i>retro</i> -retinol
CBP	carotenoid binding protein	HSA	human serum albumin
CCBP	cellular β -carotene-binding protein	Htr	methyl accepting protein
CCD	carotenoid cleavage dioxygenase	HWE	Horner–Wadsworth–Emmons
CCE	carotenoid cleavage enzyme	HY5	elongated hypocotyl 5 transcription factor
CD	circular dichroism	IBX	<i>o</i> -iodoxybenzoic acid
CD36	thrombospondin receptor	IC	internal conversion
CHl	chlorophyll	ICC	iterative cross-coupling
Chr	channel rhodopsin	Iso-A2E	N-13- <i>cis</i> -retinylidene-N-retinylethanolamine
CI	conical intersection	GPCR	G-protein coupled receptors
COP	constitutively photomorphogenic 1 (a ubiquitin ligase)	IGFR	insulin-like growth factor receptor
COX	cytochrome oxidase	ISX	gut homeodomain transcription factor
CRABP	cellular retinoic acid binding protein	KHMDS	potassium bis(trimethylsilyl)-amide
CRBP-I	cellular retinol-binding protein type I	KDM	histone demethylase
CRTISO	carotenoid isomerase	LA	light-adapted
CrtM	dehydrosqualene synthase	LAH	lithium aluminum hydride
CTCL	cutaneous T-cell lymphoma	LBD	ligand-binding domain
CYP	cytochrome P450 family of hemoproteins	LBP	ligand-binding pocket
CuTC	copper thiophene carboxylate	LDL	low-density lipoprotein
DA	dark-adapted	LHC	light-harvesting antenna complex
DBD	DNA-binding domain	LDA	lithium diisopropylamide
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene	LRAT	lecithin:retinol acyl transferase
DCC	dicyclohexylcarbodiimide	LSD1	lysine specific demethylase 1
DFT	density functional theory	LUXEA	lutein xanthophyll eye accumulation
DHA	docosahexaenoic acid	LXR	liver X receptor
DHP	dihydropyridine	MAPK	mitogen activated protein kinase
13,14-DHR	13,14-dihydroxyretinol	MCPBA	<i>m</i> -chloroperoxybenzoic acid
DIAD	di-isopropyl azodicarboxylate	MDR	medium-chain dehydrogenases
DIBAL-H	diisobutylaluminum hydride	MIDA	N-methylimidodiacetic acid
DMA	dimethylacetamide	MMTV	mouse mammary tumor virus
DMAP	<i>N,N</i> -4-dimethylaminopyridine	MNU	N-methyl- <i>N</i> -nitrosurea
DMP	Dess–Martin periodinane	MOM	methoxymethyl
DMPU	<i>N,N</i> -dimethyl propylene urea	MR	middle rhodopsin
dvds	divinyltetramethylsiloxane	NBD	nucleotide-binding domain 1
DWARF27	carotene isomerase D27	NCS	N-chlorosuccinimide
ϵ LCY	ϵ -carotene synthase	NCED	9- <i>cis</i> -epoxycarotenoid dioxygenase
EET	excitation-energy transfer	NF κ B	nuclear factor κ B
EPR	electron paramagnetic resonance	NMO	<i>N</i> -methylmorpholine <i>N</i> -oxide
ER	estrogen receptor	NMP	<i>N</i> -methylpyrrolidinone
FDA	U.S. Food and Drug Administration	eNOS	endothelial nitric oxide synthase
5-FU	5-fluorouracil	iNOS	inducible nitric oxide synthase
GDP	guanosine diphosphate	NPQ	nonphotochemical quenching
GSTP1	glutathione S-transferase	NR	nuclear receptor
GTP	guanosine triphosphate	OCP	orange carotenoid protein
HCC	hepatocellular carcinoma	OSM	oxidation state modification
HDL	high-density lipoprotein	PAH	polycyclic aromatic hydrocarbons
HKR	histidine kinase rhodopsin	PCC	pyridinium chlorochromate
		PCP	peridinin–chlorophyll complex

PDB	protein data bank	TFP	tris(2-furyl)phosphine
PDE	phosphodiester	Tf ₂ O	triflic anhydride
PE	phosphate ester	TM	transmembrane
PM	purple membrane	TMEDA	N,N,N',N'-tetramethylethylene-diamine
PML/RAR α	promyelocytic leukemia/retinoic receptor alfa fusion protein	TPAP	tetra-n-propylammonium per-ruthenate
PML	polymorphonuclear leucocytes	TRAIL	tumor necrosis factor-related apoptosis inducing ligand
PhNTf ₂	N-phenyltriflimide	TTNPB	4-[<i>(E)</i> -2-(5,6,7,8-tetrahydro-5,5,8, 8-tetramethyl-2-naphthalenyl)-1-propenyl]benzoic acid
PPARS	peroxisome proliferator-activated receptors (NR1C1, 2, and 3)	TTR	transthyretin (transporter of thyroxin and retinol)
PPRE	peroxisome proliferator-activated receptor response element	VDE	violaxanthin de-epoxidase
PPTS	pyridinium <i>p</i> -toluenesulfonate	VEGFR2	vascular endothelial growth factor receptor
PR	protorhodopsin	VITAL	vitamins and lifestyle
PS	photosystem	XBP	xanthophyll-binding protein
PSB	protonated Schiff base	ZDS	ξ -carotene desaturase
PSY	phytoene synthase	ZEP	zeaxanthin epoxidase
PT	phenyl tetrazolyl	Z-ISO	ξ -carotene isomerase
PTC	pertussis toxin		
QM/MM	quantum mechanics/molecular mechanics		
RAE	retinol activity equivalent		
RALDH	retinaldehyde dehydrogenases		
RAR	retinoic acid receptor		
RBP4	serum retinol binding protein		
RCT	randomized controlled trial		
RdCCD	<i>Rosa damascena</i> carotenoid cleavage dioxygenase		
RDH	retinol dehydrogenase		
Red-Al	sodium bis(2-methoxyethoxy)-aluminum hydride		
RetSat	retinol saturase		
Rh	rhodopsin		
ROS	rod outer segment; reactive oxygen species		
RPE	retinal pigment epithelium		
RPE65	retinal pigment epithelium 65 kDa protein		
RXR	retinoid X receptor		
SAD	Sharpless asymmetric dihydroxylation		
SCF	self-consistent-field		
SMD	short-chain dehydrogenases		
SNP	single-nucleotide polymorphisms		
SNuRM	specific NR modulator		
SR	sensory rhodopsin		
SR-B1	scavenger receptor class B type 1		
Stra6	stimulated by retinoic acid 6		
StRD	steroidogenic acute regulatory domain		
TBAF	tetrabutyl ammonium fluoride		
TBHP	tert-butyl hydroperoxide		
TBME	tert-butyl methyl ether		
TCDI	1,1'-thiocarbonyldiimidazole		
TDDFT	time-dependent density functional theory		
TDMPP	tris(2,6-dimethoxyphenyl)-phosphine		
TES	triethylsilyl		

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