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Fluorinated Retinoids and Carotenoids

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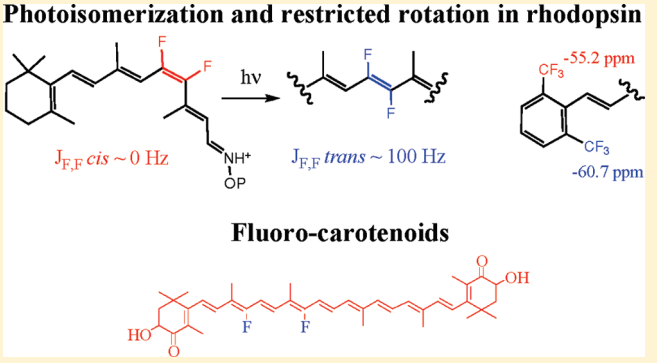
ABSTRACT: Methods of preparing fluorinated retinoids with labels located on odd-numbered carbons as well on even-numbered carbons and those containing trifluoromethyl groups are reviewed. The use of such retinoids in studies of protein-bound species is summarized, including the application of ^{19}F NMR spectroscopy for elucidating the mechanism of *cis/trans* isomerization, restricted rotation within the protein binding pocket, and identification of specific protein−substrate interactions. The fluorine label was also useful for wavelength attenuation of protein-bound species (including formation of NIR absorbing pigments) and for other unique applications. The more limited studies available on fluorinated carotenoids are also reviewed.

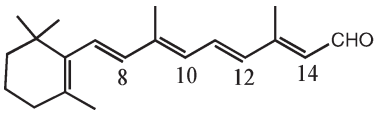


The fluorine atom is a useful NMR label for providing structural information related to protein substrate interactions in regions normally not provided by isotopic labels of natural systems (projecting beyond the substrate molecule), yet it is sufficiently compact in size to be a creditable substituent for a H-atom. Even though the high electronegativity of the fluorine label sometimes alters some of the properties of the substrate, when used properly, it might impart desirable new properties that can be harvested in a productive way. In this paper, a review of works involved in the use of F-labels in studies of retinoids and carotenoids is presented.

FLUORINATED RETINOIDS

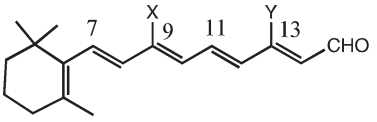
Preparation of Fluorinated Retinoids. *Vinylic F-Retinoids.* The Machleidt procedure starting from bromofluoroacetate[1] has been the most common procedure for preparing vinylic fluororetinoids. For example, the preparation of 12-fluororetinal, **1c**,[2] is shown below, which incorporates chain-elongation steps typical in retinoid synthesis (Scheme 1):[3]

Similarly, 8-F, 10-F, 14-F (**1a**,**b**,**d**), and several difluororetinals (**1e**,**f**) with labels located on even-numbered carbon atoms[4] were reported. The synthetic sequences were generally non-stereospecific. The desired 11-*cis* isomer for rhodopsin analogue preparation or the all-*trans* isomer for bacteriorhodopsin analogue preparation either was available from the synthetic mixtures or could be prepared by photoirradiation followed by isolation by preparative HPLC.



1a: 8F
1b: 10F
1c: 12F
1d: 14F
1e:10F, 14F
1f: 8F, 12F

A variation of the above procedure, where the F-label was first introduced to the C_5 or C_7 reagent before assembling the pentaene chromophore, allowed the preparation of retinoids with the F-label located at the odd-numbered carbon atoms, **2**.[5]

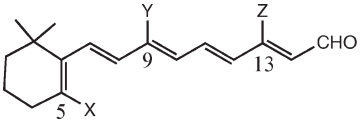


2a: X = F. Y = CH_3
2b: 11F, X = Y = CH_3
2c: X = CH_3, Y = F

A clever usage of the DAST reagent allowed preparation of vicinally disubstituted fluorinated synthons[6] that eventually afforded the desirable 11-*cis*-11,12-difluororetinal, **3**, and the related 13,14-difluororetinal (Scheme 2):[7]

Trifluoromethylated Retinoids. Trifluoromethylacetone is the common starting material for preparation of trifluoromethylated retinoids.[8] The preparation of 13-trifluororetinal (**4a**) is outlined below (Scheme 3).[9a] Interestingly, the CF_3-13 substituent reverses the relative stability of the all-*trans* and 13-*cis* isomers.[9]

9-Trifluoromethyl retinoids (**4b**) can be prepared by a similar procedure,[9] while that for 5-trifluororetinoids (**4c**) has involved a more complex procedure requiring preassembling of the labeled ring system.[10]



4a: X = Z = CH_3, Y = CF_3
4b: X = Y = CH_3, Z = CF_3
4c: Y = Z = CH_3, X = CF_3

Others. Several other ring-modified retinoids containing F-labels are also known. A few selected ones (**5**, **6**, **7**) are

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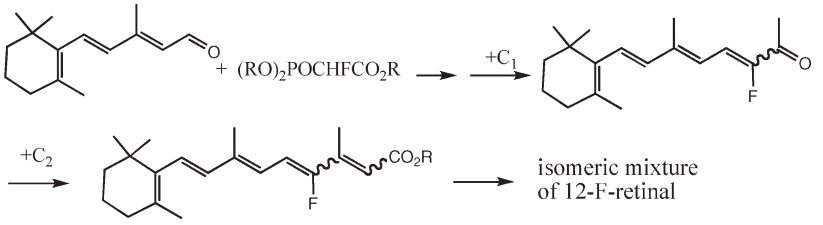
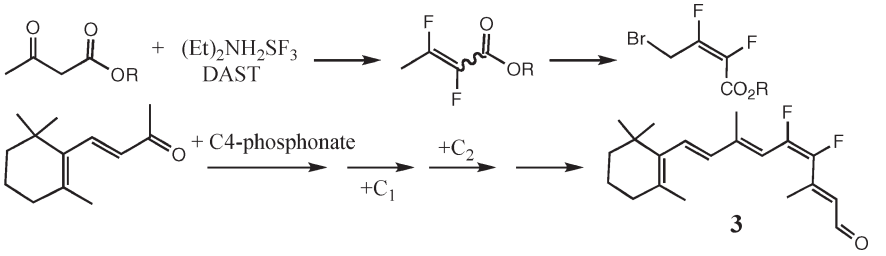
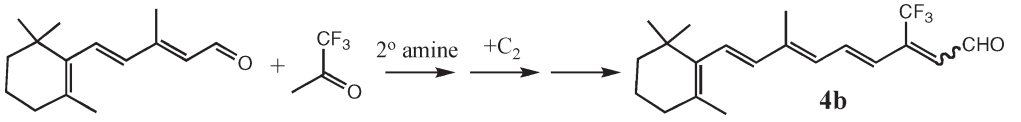
Scheme 1



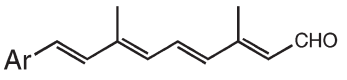
Scheme 2



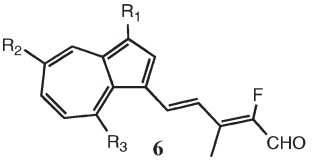
Scheme 3

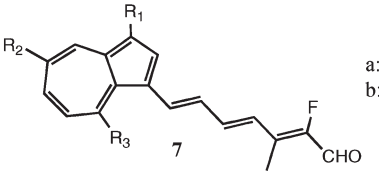


shown.[4b] Their synthesis took advantage of the many available fluoro-aromatic compounds or others that can be prepared from standard fluorinating procedures (see below).



5a: Ar = C_6F_5
5b: Ar = o-F,o'-CF_3-Ph
5c: Ar = o-CF_3,o'-CF_3-Ph
5d: Ar = o-F',o'-Cl-Ph





a: R_1,R_2,R_3 = H
b: R_1,R_3 = Me, R_2 = i-Pr

Properties of Fluorine-Labeled Retinoids. *^{19}F as an NMR Label for Reporting Structural Information.* The high sensitivity of F-nuclei, the wide range of chemical shifts, and its small size (~25% larger than the H-atom) make the F-atom an excellent NMR label for structural and mechanistic studies.[11] The unusually large three-bond coupling constants can also be tapped for elucidating the photoisomerization process, an important trigger for many photoactive biopigments.

i. Detecting Light-Induced Geometric Isomerization in Photosensitive Biopigments. Cis/trans isomerization is a common primary photochemical process for many photosensitive biopigments (e.g., 11-*cis* to all-*trans* in rhodopsin for visual transduction and all-*trans* to 13-*cis* in proton pumping and energy storage in bacteriorhodopsin, bR).[12] The primary process for rhodopsin has been demonstrated with a labeled rhodopsin analogue that contained two F-atoms located at the key 11,12-positions.[7] The study took advantage of the knowledge that *cis*-vicinal F,F coupling constants are generally small (0−10 Hz), while those of *trans*-vicinal F,F coupling are large (~100 Hz).[13] 11-*cis*-11,12-Difluororetinal was found to give a rhodopsin analogue (λ_{max} 503.5 nm vs 498 nm for rhodopsin) with equal ease to the parent system. Upon irradiation of the analogue with visible light, the broad fluorine singlet signal was transformed to two broad doublets (the coupling constant being 103 Hz), clearly demonstrating the *cis* to *trans* photoisomerization process.[7]

During this study, the appearance of a fluoride ion was also detected, which seemed to be associated with substrates containing a F-atom located on the odd-numbered carbon. It is worth noting that such sites are amenable to nucleophilic addition. The subsequent fluoride elimination could lead to an interesting prospect of permanent attachment of the chromophore to the binding pocket,[7] making potentially such an F-atom an affinity-labeling functionality.

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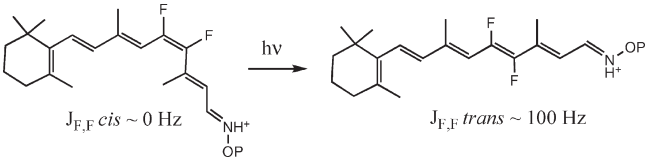
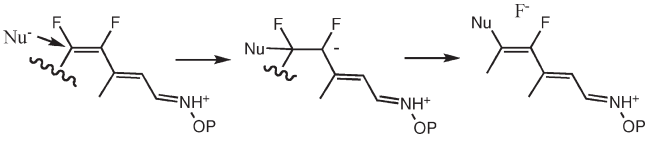
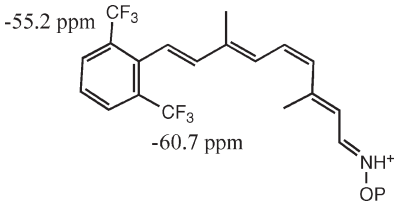
Scheme 4



Scheme 5



ii. Detecting Restricted Rotation within a Binding Pocket. When occupying the restricted space within the binding pocket of rhodopsin, the ring−chain conformational interconversion can be severely restricted. In fact, the fixed conformation is believed to be the source of the strong CD curve that is characteristic of a bound retinyl chromophore (none detectable for the free chromophore).[12] To demonstrate restricted ring−chain conformational interconversion for bound species, the Hawaii group prepared the bis-trifluoromethylphenyl rhodopsin. It exhibited two F-singlets (−55.2 and −60.7 ppm) for the two nonequivalent CF_3 groups.[14] Obviously, for the free chromophore in solution where rapid rotation around the single bond is possible, only one F-signal (−58.7 ppm) was detected. The same phenomenon was detected for the tetrafluoro analogue, **5b**.[15]



iii. Detecting Regiospecific Protein Perturbation. A more extensive study on F-rhodopsins resulted in accumulation of all F-chemical shift data of 8, 10, 12, 14, and other bis labeled fluororhodopsins. The term "F-opsin shift" (FOS), calculated from subtracting the observed chemical shift (in ppm) for the F-rhodopsin by that of the free protonated Schiff base (PSB) in solution, is a useful quantity, which reflects effects of local protein perturbation on a F-label.[16]

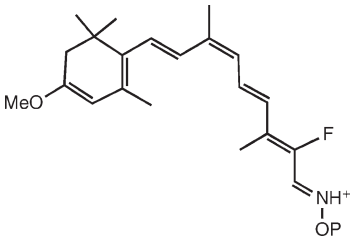
The FOS data of F-rhodopsins (11-*cis*) and the closely related (in shape) F-isorhodopsins (9-*cis*) are reproduced in Table 1. It is clear they all exhibited a positive value with most falling within the range ~4−8 ppm. An F-shift of such a magnitude is generally called a protein shift, attributable to the enclosed environment faced by a bound fluoro substrate.[11] However, there are also several values (Table 1) falling significantly outside this range: those for the 8F and 12F values for the 11-*cis* isomers. The high values of 8Fs are attributed to close contact of the F-label with the amino acid residues comprising the hydrophobic binding pocket known to exist around the ring portion of the chromophore.[12]

Table 1. FOS NMR Shift Values for F-Rhodopsins (11-*cis*) and F-*iso*-Rhodopsins (9-*cis*)

F-substituent	FOS[a] for 11-*cis* (ppm)	FOS[a] for 9-*cis*[b] (ppm)
8-fluoro	13.1	6.6
10-fluoro	4.8	4.4
12-fluoro	13.2	6.6
14-fluoro	8.4	7.9
8,12-difluoro	11.8	11.7
10,14-difluoro	3.5, 5.1	3.7, 7.6

[a] Chemical shift of the pigment in detergent minus that of the free chromophore in $CDCl_3$. [b] See refs 16 and 21.

The high-field shift of 12F was unexpected and deserved a closer examination.



The X-ray crystal structure of rhodopsin[17] showed that C-12 of the retinyl chromophore is in close proximity with the carbonyl group of Cys-187. In fact, for the calculated structure of the 11-*cis*-retinyl chromophore, H-12 and O of the carbonyl group of Cys-187 are within vdW contact.[17] Thus, the observed high-field shift of 12F is likely a result of steric depolarization, i.e., a displacement of electron density away from the F-reporter toward the carbon framework[16] as a result of the steric crowding. This type of deshielding effect is known in congested model organic compounds, such as 1,8-disubstituted naphthalenes.[18] This piece of information derived from NMR studies not only was a confirmation of the crystal structural work for a region where the exact H-atomic positions are difficult to obtain but also supports nicely the unique deuterium isotope effect detected by resonance Raman studies during decay of photoexcited 12-D-rhodopsin.[19]

Since photoexcitation is expected to cause an overall bond elongation for the π-chromophore,[20] stimulation by light should further increase compression near H-12.[21] This light-induced extra protein−substrate steric compression is believed to be the cause for the unusually high photosensitivity of rhodopsin.[20]

Due to difficulties in preparing high concentration samples of bR crystal in detergent solution (and unfavorable F-frequency for solid sample studies), few F NMR studies have been performed with fluorinated bR analogues.[22]

Modified Properties in Protein−F-Retinoid Interactions. The unusually large electronegativity of the F-atom can lead to unusual stability (or the lack of it) to a substrate. Especially pronounced is the polar effect on the stability of the protonated Schiff bases (PSB) of retinoids.

i. Wavelength Attenuation Promoted by F-Substituents. The chromophore for a retinal binding protein usually exists in the form of a PSB.[12] Any substituent that stabilizes the ground-state structure of PSB should lead to a blue-shift, while that of destabilized PSB should lead to a red-shift. This has been amply

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Table 2. Absorption Maxima of Visual Pigments (11-*cis* and 9-*cis*) and Bacteriorhodopsin Analogues

F-substituent	rhodopsin (11-*cis*) (nm)	isorhodopsin (9-*cis*) (nm)	bR (all-*trans*) (nm)
8-fluoro[a]	463	460	530[b]
10-fluoro[a]	489	486	565[b]
12-fluoro[a]	507	493	591[b]
14-fluoro[a]	527	511	587, 680[b]
14-chloro[b]			691[b]
9-fluoro[c]			518
11-fluoro[c]			540
13-fluoro[c]			548
11,12-difluoro[d]	503.5		
5-trifluoromethyl[e]	457	454	
9-trifluoromethyl[e]	456	447	
13-trifluoromethyl[e]	542	516	624[f]

[a] Data from ref 4a. [b] Data from ref 24. [c] Data from ref 5. [d] Data from ref 7. [e] Data from ref 4b. [f] From ref 9a, however, probably that of the more stable 13-*cis* (see ref 9b).

demonstrated in the series of fluorinated rhodopsins, isorhodopsins, and bacteriorhodopsins.[3]

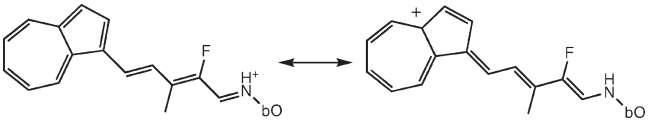
F-Substituents located at even-numbered carbon atoms are not in a position to participate in resonance stabilization of the delocalized iminium ion. Instead, the highly distant sensitive inductive effect will take over. Thus, when the F-atom is located near the iminium N, its high electronegativity destabilizes the cation with the expected red-shift of the long-wavelength absorption band, as shown in both rhodopsin and bR analogues (Table 2). This effect diminishes when the F-substituent is located farther away from the iminium N-atom. In an attempt to prepare a very red-shifted rhodopsin analogue, the Hawaii group prepared one containing a 3-methoxy group on an extra 3,4-double bond for extended delocalization of the positive charge, further enhanced by the presence of the 14-F-substituent.[23] Its absorption maximum (653 nm) is considerably red-shifted from those of rhodopsin (498 nm) and porphyropsin (another native visual pigment with a 3-dehydroretinal chromophore, 505 nm), albeit with much diminished G-protein activation efficiency.[23]

For bacteriorhodopsin (λ_{max} = 568 nm), the 14-F and 14-Cl analogues showed absorptions at 680 and 691 nm,[24] and some of the F-azulenic analogues (from azulenic retinal analogues **6** and **7**) exhibited absorptions in the NIR region (>700 nm).[25] However, such NIR absorbing pigments no longer possess the characteristic proton-pumping activity of bR.[26]

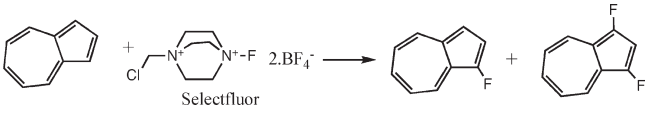
The unexpectedly large perturbation observed for the F-azulenic bacteriorhodopsin analogue is highly dependent on the ring−chain substitution pattern. For the NIR absorbing pigments, the chain portion of the chromophore must be located on an odd-numbered carbon of the azulene chromophore rather than an even-numbered one, so as to allow delocalization of the positive charge to form the stable 6*e* aromatic tropylium ion (cross-conjugated structures for 2-substituted analogues),[27] as shown in the structures below. When an additional F-atom is introduced at the "14-equivalent" position, an unusually red-shifted pigment (>750 nm) was obtained. (The binding site of bR is more flexible than that of rhodopsin. Thus, the more bulky guaiazulene analogue **7** also gave red-shifted bR analogues.)[25,28]

ii. Effect of the 10F-Substituent in Rhodopsin. During a study of the decay kinetics following photoexcitation of 10-fluororhodopsin, features different from those of the parent rhodopsin were detected. It was postulated that the observed perturbation was due to a specific electrostatic interaction between the host and the 10F-substituent.[29] Such an unusual perturbation was also detected in a more recent biochemical and FT-IR study of 10-F-rhodopsin in comparison with other fluororhodopins.[30] In fact, 10-F-rhodopsin was found to possess a much reduced signaling activity.[30]

Scheme 6



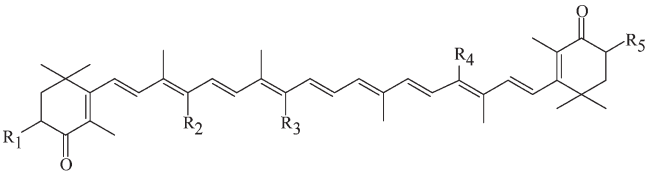
Scheme 7



iii. Facilitating Preparation of F-Azulenic Compounds. A side issue in studies of azulenic polyene chromophores was the finding that the five-membered ring of the bicyclic azulene was sufficiently strong as a nucleophile to react with Selectfluor, a relatively new fluorinating reagent that provides an F^+-equivalent. A series of 1-fluoro and 1,3-difluoro azulenes and derivatives were prepared in fair yields in a one-step reaction.[31] Such products turned out to be useful in preparing chromophores with unusually large fluorescence yields from an unusually long lived second singlet state of the chromophore.[32]

FLUORINATED ASTAXANTHINS AND RELATED CAROTENOIDS

Fluorinated Astaxanthins. A few F-labeled carotenoids were synthesized for studies of carotenoproteins. α-Crustacyanin, the blue astaxanthin−protein complex, was isolated from the carapace of the lobster *Homarus gammarus*. The unique color change from red to blue, while synthetic astaxanthins (**8a**) associate noncovalently with the apoproteins isolated from the natural α-crustacyanin,[33] prompted the Hawaii group to prepare some fluorinated astaxanthins for better understanding of the properties of carotenoproteins.



8a: R_1=OH, R_2=R_3=R_4=H, R_5=OH;
8b: R_1=OH, R_2=F, R_3=R_4=H, R_5=OH;
8c: R_1=OH, R_2=F, R_3=H, R_4=F, R_5=OH;
8d: R_1=H, R_2=F, R_3=R_4=H, R_5=OH;
8e: R_1=OH, R_2=H, R_3=F, R_4=H, R_5=OH;
8f: R_1=F, R_2=R_3=R_4=H, R_5=F.

In 1997, all-*trans* 10-F- (**8b**), 10,10′-F_2-astaxanthin (**8c**), 10′-F-adonirubin (**8d**), and 14-F-astaxanthin (**8e**) were prepared

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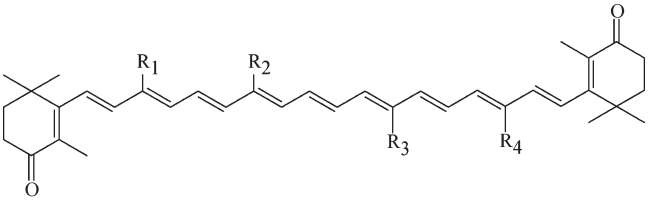
using the $C_{15} + C_{10} + C_{15}$ synthetic approach normally applied for the synthesis of carotenoids. The Wittig−Horner condensation of the fluorinating C_2-phosphonoester reagent was used for the preparation of the 10-fluoro-C_{15} intermediate and 3-fluoro-C_{10}-dialdehyde, which were necessary components for the synthesis of 10-fluorinated analogues and 14-F-astaxanthin, respectively.[34] All-*trans* isomers of the four fluorinated analogues were isolated and then reconstituted successfully into the natural apoproteins to yield the corresponding crustacyanin pigment analogues.[35] In 2002, 3,3′-F_2-canthaxanthin (**8f**) was prepared by first converting the 3- and 3′-hydroxy groups of all-*trans* astaxanthin into a bis-triflate, followed by a further change of the bis-triflate into 3,3′-F_2 upon treatment with 2 equiv of *n*-Bu_4NF in THF.[36] 3,3′-F_2-Canthaxanthin was also combined with the apoproteins to give a blue α-crustacyanin analogue. In the same study, other 3,3′-bis-halogenated canthaxanthins were also prepared.

UV−vis absorption spectra of the fluorinated analogues of α-crustacyanin showed a similar shape to that of natural α-crustacyanin, and the blue-shifted absorption maxima of the analogues were observed.[35] The UV−vis spectroscopic data of the fluorinated astaxanthins and the crustacyanin analogues suggested that the fluorinated astaxanthin analogues are bound in the same way as natural astaxanthin. The blue shifts are probably due to the effects of the electronegative fluorine atom.

$A^{19}F$ NMR study of α-crustacyanin pigment analogues from 10-F-astaxanthin, 10,10′-F_2-astaxanthin, and 10-F-adonirubin was also conducted.[35] A downfield shift of the fluorine signal at −111 ppm, which is typical of that of a pigment when it is bound with proteins, was observed for the three analogues. The three similar broad signals suggest that the 10-F region may not be the critical binding site, which is responsible for the significant color change. The α-crustacyanin pigment analogues from 14-F-astaxanthin and 3,3′-F_2-canthaxanthin were relatively unstable, and the fluorine spectra were not obtainable. The unsuccessful F NMR study of the latter analogues of α-crustacyanin may indicate the unusual noncovalent interactions between astaxanthins and the apoproteins. Future studies in this difficult area are certainly needed.



9a: R_1=R_2=R_3=R_4=H;
9b: R_1=CF_3, R_2=R_3=R_4=H;
9c: R_1=H, R_2=CF_3, R_3=R_4=H.

Trifluoromethylated *β*-Carotene and Canthaxanthin (9a). Several trifluoromethylated carotenoids such as CF_3-9-, CF_3-13-, 9,9′-bis-CF_3-, and 13,13′-bis-CF_3-*β*-carotene and CF_3-9- (**9b**) and 13-CF_3-canthaxanthin (**9c**) were reported in the literature.[37] The CF_3 group was mainly introduced by reaction of a C_{15} or C_{20} Wittig salt with a 9-CF_3- or 13-CF_3-aldehyde. The CF_3 group appears to have a strong *cis*-directing effect, giving the major *cis* isomers in the synthetic mixtures. Sensitized irradiation of the mixtures increased the amount of all-*trans* isomers, which were found to be unstable at room temperature in some cases. Electrochemical properties of these trifluoromethylated carotenoids were also investigated in relation to other electrochemical studies of carotenoids.[38]

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DEDICATION

Dedicated to Dr. Koji Nakanishi of Columbia University for his pioneering work on bioactive natural products.

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