Absolute configurational assignment of 3-hydroxycarotenoids

Thomas Andersson,[a] Babak Borhan,[a]† Nina Berova,[a] Koji Nakanishi,*[a] Jarle Andre Haugan[b] and Synnøve Liaaen-Jensen[b]

[a] *Department of Chemistry, Columbia University, New York, NY 10027, USA*
[b] *Organic Chemistry Laboratories, Department of Chemistry and Biology, Norwegian University of Science and Technology, N-7491 Trondheim-NTNU, Norway*

Received (in Cambridge, UK) 15th March 2000, Accepted 30th March 2000
Published on the Web 30th June 2000

Attempts were made to develop an exciton chirality method applicable to the unique cases represented by 3-hydroxycarotenoids. However, this approach has so far not been successful. The 3-hydroxy configurations were therefore determined by the extended Mosher ^{1}H-NMR method. Nine carotenoids with seven different end groups and of known 3-hydroxy configurations were derivatized with (*R*)- and (*S*)-methoxyphenylacetic acid (MPA); they all gave the expected shift differences. Three other auxiliary reagents with naphthalene and anthracene nuclei gave larger and consistent NMR shift differences, but they are not yet commercially available.

Introduction

X-Ray analysis has so far not been suited for stereochemical assignments of chiral carotenoids due to difficulties involved in crystallization.[1] Stereochemical correlations have thus been based on certain key carotenoid oxidation products with configurations established by X-ray crystallography or by total synthesis of optically active carotenoids.

Comparative CD spectroscopy, often in combination with ^{1}H-NMR data, has served as the most useful tool for establishing the absolute chirality of naturally occurring carotenoids. However, the current CD approach suffers from the disadvantage that: (i) conjugation is essential between the chiral end group and the polyene chromophore; (ii) *Z*-configuration in the polyene chain inverts the Cotton effect in the case of certain end groups; (iii) chiral allenic end groups (with two chiral centers and one chiral axis) and chiral acetylenic end groups give rise to only weak Cotton effects; and (iv) in carotenoids with 5,6-epoxy or ε- end groups, the effect of the 3-hydroxy group is practically nil.[2,3]

Of the *ca*. 600 fully or partly characterized naturally occurring carotenoids, *ca*. 60% are chiral.[4] Of these about 300 are secondary alcohols, the hydroxy function most frequently being present in substituted cyclic β- and ε- or aliphatic ψ-end groups.[5]

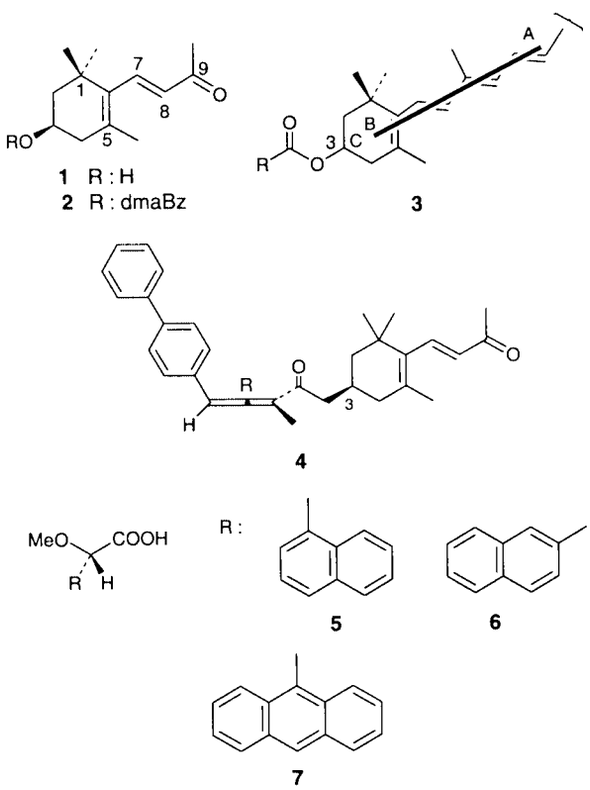

Chart 1

Results and discussion

Attempted application of the exciton chirality method

Using (3*R*)-3-hydroxy-β-ionine (**1**),‡ λ_{max} (EtOH) 280 nm (ε 8200) as a model, we attempted to apply the exciton chirality method[6,7] by acylating the 3-hydroxy with a chromophore, *e.g.*, *p*-dimethylaminobenzoic acid (dmaBz) λ_{max} (EtOH) 309 nm (ε 30 4000), that would couple with the existing dienone chromophore (Chart 1). β-Ionone dmaBz (**2**) did indeed give a negative couplet with a moderate CD amplitude ($A = -8.3$), 303 nm (−5.3)/273 nm (+3.0) (in hexane), thus showing that the electric transition moments of the two chromophores constitute a negative twist.[8] However, it turns out that the unique structure of the 3-hydroxy-β-ionine molecule does not allow establishment of the 3-hydroxy configuration. This is because of the flexibility of the cyclohexene ring, and the uncertainty of the side-chain conformation around the 6-s-*cis* bond as evidenced by the multiple NOEs detected between 7-H/1-Me, 7-H/5-Me, 7-H/9-Me, 8-H/1-Me, and 8-H/5-Me.[9] However, the overriding reason is that the two chromophores are located "*para*" across the cyclohexane ring and that the two chromophoric electric transition moments lie in almost the same plane

† *Present address:* Department of Chemistry, Michigan State University, East Lansing, MI 48824, USA.
‡ The IUPAC name for compound **1** is 4-(4-hydroxy-2,6,6-trimethylcyclohex-1-en-1-yl)but-3-en-2-one.

DOI: 10.1039/b002088p

Table 1 Shift differences $\delta_{(R)} - \delta_{(S)}$ (ppm) of MPA esters of 3-hydroxy compounds

Compound	gem-diMe[e]	2-Hax	2-Heq	4-Hax	4-Heq	5-Me
1[b]	−0.048	−0.098	−0.137	+0.177	+0.139	+0.061
8[b]	−0.044	−0.096	−0.138	+0.173	+0.137	+0.056
9[b]	−0.050	−0.102	−0.16[a]	+0.17[a]	+0.136	+0.054
10[b]	−0.047	−0.101	−0.140	+0.173	+0.139	+0.055
11[b]	−0.034	−0.143	−0.135	n/a	n/a	+0.032
12[b]	−0.033	−0.10[a]	−0.14[a]	+0.08[a]	+0.13[a]	+0.054
13[c]	+0.023	+0.085	+0.074	n/a	n/a	−0.031
14[c]	−0.058	−0.085	−0.101	+0.111	+0.099	+0.064
15[c]	−0.025	−0.078	−0.086	n/a	n/a	+0.026
16[c,f]	−0.045	−0.097	−0.140	+0.172	+0.137	+0.056
16[c,g]	+0.119	+0.128	+0.233	n/a	−0.142[d]	−0.061

[a] Estimated from ^{1}H–^{1}H COSY spectra. [b] Solvent $CDCl_3$. [c] Solvent C_6D_6. [d] Vinyl proton. [e] Average shift difference. [f] (β-3OH side). [g] (ε-3OH side).

(see **3**). Depending on the rotation around the 6,7 bond and conformation of the cyclohexene ring, the chirality between the transition moments of the side chain and the 3β-chromophore can either be negative or positive. This situation in encountered in the 3-hydroxy carotenoids as well. For this reason, although the Trondheim group prepared several carotenoid 3-retinoates,[10] unfortunately the CD results were not conclusive.

Further CD tests revealed that insertion of a twisted auxiliary chromophore exemplified by (R_a,3*R*)-allenic β-ionone ester (**4**) did give rise to a negative couplet around 275 nm, $A = ca.\ -28$, associated with interaction between the biphenyl and ionone chromophores.[8] This approach was conceptually useful, but difficulties in handling the reactive allenic esters, especially at microgram levels, made it unsuited for the present carotenoid case. However, attempts to devise a microscale CD method applicable to cases represented by 3-hydroxy-β-ionine are still being pursued.

The Mosher method

In the following we report a systematic study on the application of the extended Mosher method as an alternative for absolute configurational assignment of 3-hydroxycarotenoids. This derivatization/^{1}H-NMR protocol does not suffer from the mentioned CD related limitations and can be performed with 200 microgram level samples. Nine carotenoid models of known 3-hydroxy configuration with seven different end groups were selected for this study (Fig. 1).

Determination of the absolute configuration of chiral organic compounds by the Mosher method[11] and extensions[12,13] is performed by reacting the compound with two enantiomeric auxiliary reagents and evaluating the shift difference between the two derivatives; configurations of primary and secondary alcohols and amines and acids have been determined by this method.[14] As depicted in Fig. 2, depending on the chirality of the auxiliary reagent, the aromatic nuclei specifically shield one side of the chiral compound. This gives rise to shift differences of opposite signs between the two diastereomeric derivatives. For secondary alcohols, the frequently used auxiliary reagent is the commercially available methoxyphenylacetic acid (MPA).[12] A conformational study of the ester bond formed in MPA derivatives has shown that the carbonyl oxygen is synperiplanar to both the methine hydrogen and the methoxy group, and that the phenyl group and the C–H bond in MPA reside in the same plane.[15]

The applicability of the method was tested with (3*R*)-3-hydroxy-β-ionone (**1**) synthesized as reported earlier.[16] The ionone **1** was converted into the corresponding (*S*)- and (*R*)-MPA esters by reaction with excess (*S*)- and (*R*)-MPA in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and 4-dimethylaminopyridine (DMAP).[12] A comparison of the shift differences between the two diastereomeric MPA esters of **1** confirmed that the Mosher method could be used for determining the absolute configuration of 3-hydroxycarotenoids (Table 1, entry 1).

Auxiliary chromophores other than MPA, namely chromophores containing 1- and 2-naphthyl and 9-anthryl moieties (Chart 1, **5**, **6**, **7**) were also checked with 3-hydroxy-β-ionone (Chart 1).[17] Since however, only the (*R*)-enantiomers of these acids were available, the induced NMR shifts were estimated after separation of the two diastereomeric esters derived from the racemic ionone. The largest shift differences were induced by the 9-anthryl group, which was followed by 1-naphthyl and then the 2-naphthyl reagents; the shifts were up to several-fold that of MPA, which exhibited the smallest shifts. However, the MPA esters of 3-hydroxycarotenoids yielded satisfactory shift differences for making reliable determinations. Furthermore, MPA is currently the only commercially available compound.

All determinations of the 3-hydroxy configuration of carotenoids by the Mosher method gave results consistent with their known absolute configurations. The configuration of all model carotenoids studied here has been established by methods including total synthesis of the optically pure carotenoids. References for the synthesis of each compound are shown after the compound number. The compounds used in the present studies (Fig. 1) ranged from polyene 3-hydroxycarotenoids such as zeaxanthin **8**[18] to more complex structures containing allenes as in paracentrone **12**,[19] triple bonds as in the apocarotenoid **9**,[20] triophaxanthin **10**,[21] apohopkinsiaxanthin **11**[22] and tetradehydroastaxanthin **15**,[23] ketones in **11**,[22] astaxanthin **13**,[24] tetrahydrozeaxanthin-8,8′-dione **14**[25] and **15** and a *Z*-double bond in **11**. In some cases the compounds had opposite chirality or two different chiral hydroxys as in astaxanthin **13** and lutein **16**.[26] Note that the induced shifts differ only slightly among the various carotenoids in Table 1. In cases where shift differences are observed, it is because the presence of functional groups such as ketones (**11**, **13**, **15**), allenes (**12**) or an ε-OH group (**16**), might lead to conformational changes in the six membered ring.

Quinoa *et al.* reported a method using only one MPA-derivative and measuring the shift differences at two different temperatures.[27] At room temperature MPA is believed to consist of two conformers that shield different sides of the chiral molecule. One of the conformers is thermodynamically more stable and more abundant at room temperature. This conformer becomes more populated upon lowering the temperature, thus enhancing the shielding effect on one side, while decreasing the shielding effect from the other conformer. This results in increased chemical shift difference between the two, hence leading to establishment of absolute configuration. To check this method, (*R*)-MPA was reacted with (*R*,*R*)-zeaxanthin and the ^{1}H-NMR spectrum was taken at room temperature and at 215 K. However, in the present case the shift differences between these two temperatures were all positive and hence not in agreement with the proposed protocol.

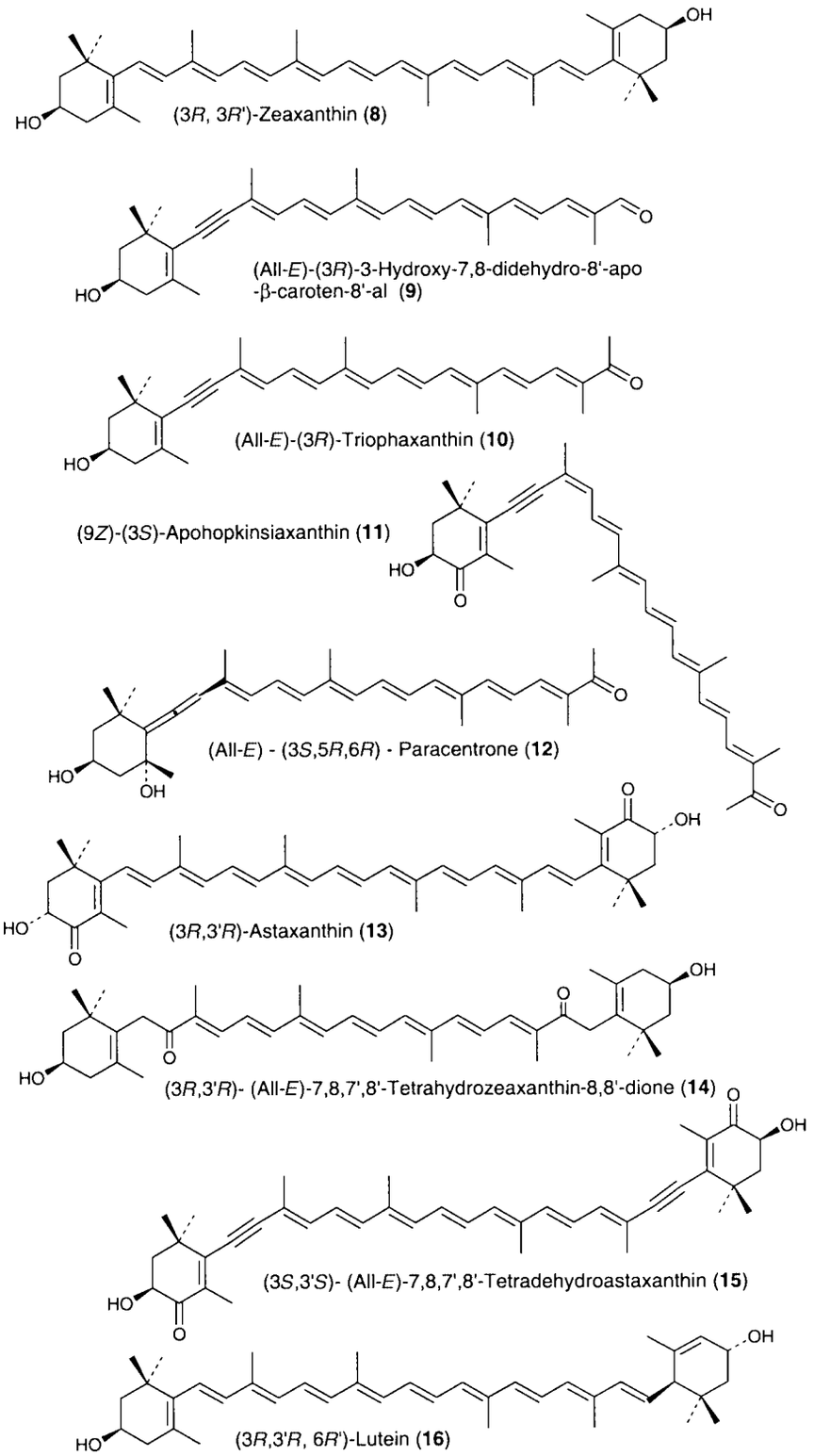

Fig. 1 Structure of carotenoids **8**–**16**.

Conclusion

The results demonstrate the usefulness of the NMR Mosher method for assigning the absolute configurations of a variety of 3-hydroxycarotenoids with different chain structures and end groups. The protocol can be performed at the microgram level including derivatization. *Z*-Bonds in the polyene chain do not affect the results, and conjugation of the chiral end group with the polyene chain, such as zeaxaanthin **8**, is not required. Also, carotenoids with weak Cotton effects give the correct assignment by this method. For lutein **16** the present results represent a different and additional assignment from the previous determination of the 3′*R*-configuration which was based on partial synthesis and CD.[28,29]

Experimental

General experimental procedures

All compounds were purchased from Aldrich except the 3-hydroxycarotenoids, which were synthesized (lutein **16** was isolated from alfalfa). Methylene chloride was distilled from calcium hydride under nitrogen. Analytical and preparative HPLC was performed on Waters (Rochester, MN) or Rainin

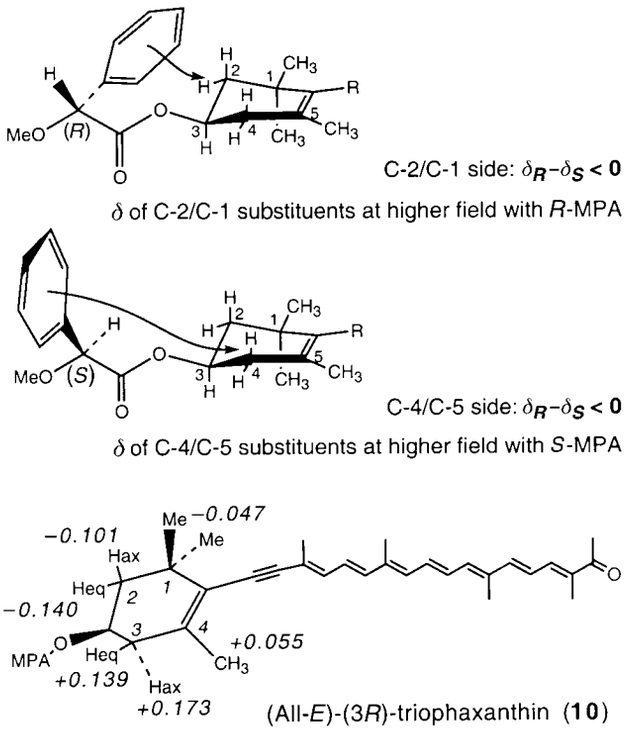

Fig. 2 Expected NMR shifts in the diastereomeric esters of methoxyphenylacetic acid (MPA) and the actual shifts observed in ester **10** in $CDCl_3$.

(Woburn, MA) HPLC. The diastereomeric mixtures obtained by reaction of racemic 3-hydroxy-β-ionone with (*S*)-MPA, (*R*)-1-NMA (**5a**, **5b**), (*R*)-2-NMA (**6a**, **6b**) and (*R*)-9-ATMA (**7a**, **7b**) were separated using a chiral column (Chiracel OD), 1 : 99 propan-2-ol–hexane, $\lambda = 280$ nm. ^{1}H and ^{1}H–^{1}H COSY spectra were recorded on a Bruker 500 DMX MHz spectrometer and performed in $CDCl_3$ or C_6D_6. Chemical shifts (δ) are reported in ppm downfield from internal TMS and coupling constants (J) in Hz. ESI mass spectra were measured on a JEOL JMS-mate LSMS-system. $[a]_D$ values were measured on a JASCO DIP-1000 polarimeter and are given in 10^{-1} deg cm^2 g^{-1}. Preparative TLC-plates used were Uniplate Silicagel GF 20 × 20 cm 500 or 1000 microns, and they were bought from Analtech Inc.

General esterification procedure

The alcohol (normally 0.25 mg, 1 eq.), excess auxiliary acid (>10 eq.) and excess EDC (>10 eq.) were dissolved in *ca.* 1 ml dry CH_2Cl_2 and DMAP (1 eq.) was then added to the solution and stirred at room temperature for 5–30 min. The reaction was followed on TLC (*ca.* 30% ethyl acetate–hexane). When the reaction was completed the reaction mixture was directly applied to and eluted (20–30% ethyl acetate–hexane) on a preparative TLC plate. Prior to NMR analysis the samples were dried in vacuum overnight.

3-Hydroxy-β-ionone (1) and its MPA esters

(*R*)-3-Hydroxy-β-ionone. The (*S*)-MPA ester of (*R*)-3-hydroxy-β-ionone (**1**) (3 mg, 8.4 μmol), was dissolved in 3 ml of methanol. Excess K_2CO_3 was added and the mixture was stirred at room temperature for 30 min. The reaction was quenched by addition of 1 ml of water and the product extracted with diethyl ether and dried with Na_2CO_3. Yield 90%. $[a]_D = -73.5$ ($c = 0.129$, 1,4-dioxane).

(*S*)-3-Hydroxy-β-ionone. The (*S*)-MPA ester of (*S*)-3-hydroxy-β-ionone (**1**) (2.5 mg, 7.0 μmol) was dissolved in 3 ml of methanol. Excess K_2CO_3 was added and the mixture was stirred at room temperature for 30 min. The reaction was quenched by addition of 1 ml of water and the product extracted with diethyl ether and dried with Na_2CO_3. Yield 90%. $[a]_D = +72.2$ ($c = 0.116$, 1,4-dioxane).

(*S*)-MPA ester of (*R*)-3-hydroxy-β-ionone. Separated from a reaction of racemic 3-hydroxy-β-ionone with (*S*)-MPA, using a chiral column (Chiracel OD), 1 : 99 propan-2-ol–hexane, $\lambda = 280$ nm. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ_H 1.09 (3H, s, 1-Me), 1.13 (3H, s, 1-Me), 1.62 (1H, t, $J = 12$ Hz, 2-Hax), 1.68 (3H, s, 5-Me), 1.81 (1H, ddd, $J = 12$, 3.5, 1.8 Hz, 2-Heq), 1.99 (1H, dd, $J = 17.5$, 9 Hz, 4-Haz), 2.29 (3H, s, 9-Me), 2.36 (1H, dd, $J = 17.5$, 5.6 Hz, 4-Heq), 3.42 (3H, s, O-Me), 4.74 (1H, s, H-MPA), 5.11 (1H, dddd, $J = 12$, 9, 5.6, 3.5 Hz, 3-H), 6.07 (1H, d, $J = 16$ Hz, 8-H), 7.16 (1H, d, $J = 16$ Hz, 7-H), 7.33–7.45 (5H, phenyl). ESI-MS: 357.3 (M + H).

(*S*)-MPA ester of (*S*)-3-hydroxy-β-ionone. Separated from a reaction of racemic 3-hydroxy-β-ionone with (*S*)-MPA, using a chiral column (Chiracel OD), 1 : 99 propan-2-ol–hexane, $\lambda = 280$ nm. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ_H 1.04 (3H, s, 1-Me), 1.10 (3H, s, 1-Me), 1.53 (1H, t, $J = 12$ Hz, 2-Hax), 1.67 (1H, ddd, $J = 12$, 3.6, 1.8 Hz, 2-Heq), 1.74 (3H, s, 5-Me), 2.17 (1H, dd, $J = 18$, 9 Hz, 4-Hax), 2.29 (3H, s, 9-Me), 2.50 (1H, dd, $J = 18$, 6 Hz, 4-Heq), 3.42 (3H, s, O-Me), 4.75 (1H, s, H-MPA), 5.10 (1H, dddd, $J = 12$, 9, 6, 3.5 Hz, 3-H), 6.09 (1H, d, $J = 16$ Hz, 8-H), 7.16 (1H, d, $J = 16$ Hz, 7-H), 7.33–7.46 (5H, phenyl). ESI-MS: 357.3 (M + H).

(*R*)-1-NMA ester of (*R*)-3-hydroxy-β-ionone (5a). Separated from a reaction of racemic 3-hydroxy-β-ionone with (*R*)-1-NMA, using a chiral column (Chiracel OD), 1 : 99 propan-2-ol–hexane, $\lambda = 280$ nm. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ_H 0.92 (3H, s, 1-Me), 1.05 (3H, s, 1-Me), 1.40 (1H, t, $J = 12$ Hz, 2-Hax), 1.57 (1H, ddd, $J = 12$, 3, 1.6 Hz, 2-Heq), 1.71 (3H, s, 5-Me), 2.14 (1H, dd, $J = 18$, 9 Hz, 4-Hax), 2.27 (3H, s, 9-Me), 2.48 (1H, dd, $J = 18$, 6 Hz, 4-Heq), 3.46 (3H, s, O-Me), 5.09 (1H, m, 3-H), 5.38 (1H, s, H-NMA), 6.05 (1H, d, $J = 16$ Hz, 8-H), 7.12 (1H, d, $J = 16$ Hz, 7-H), 7.46–8.30 (7H, 1-naphthyl). ESI-MS: 407.3 (M + H).

(*R*)-1-NMA ester of (*S*)-3-hydroxy-β-ionone (5b). Separated from a reaction of racemic 3-hydroxy-β-ionone with (*R*)-1-NMA, using a chiral column (Chiracel OD), 1 : 99 propan-2-ol–hexane, $\lambda = 280$ nm. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ_H 1.02 (3H, s, 1-Me), 1.10 (3H, s, 1-Me), 1.56 (3H, s, 5-Me), 1.56 (1H, t, $J = 12$ Hz, 2-Hax), 1.77 (1H, ddd, $J = 12$, 3, 1.6 Hz, 2-Heq), 1.83 (1H, dd, $J = 18$, 9 Hz, 4-Hax), 2.25 (1H, dd, $J = 18$, 6 Hz, 4-Heq), 2.27 (3H, s, 9-Me), 3.46 (3H, s, O-Me), 5.10 (1H, m, 3-H), 5.36 (1H, s, H-NMA), 5.99 (1H, d, $J = 16$ Hz, 8-H), 7.12 (1H, d, $J = 16$ Hz, 7-H), 7.46–8.29 (7H, 1-naphthyl).

(*R*)-2-NMA ester of (*R*)-3-hydroxy-β-ionone (6a). Separated from a reaction of racemic 3-hydroxy-β-ionone with (*R*)-2-NMA, using a chiral column (Chiracel OD), 1 : 99 propan-2-ol–hexane, $\lambda = 280$ nm. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ_H 1.01 (3H, s, 1-Me), 1.08 (3H, s, 1-Me), 1.52 (1H, t, $J = 12$ Hz, 2-Hax), 1.66 (1H, ddd, $J = 12.5$, 3, 1.8 Hz, 2-Heq), 1.73 (3H, s, 5-Me), 2.17 (1H, dd, $J = 18$, 9 Hz, 4-Hax), 2.29 (3H, s, 9-Me), 2.51 (1H, dd, $J = 18$, 6 Hz, 4-Heq), 3.46 (3H, s, O-Me), 4.91 (1H, s, H-NMA), 5.10 (1H, m, 3-H), 6.08 (1H, d, $J = 16$ Hz, 8-H), 7.15 (1H, d, $J = 16$ Hz, 7-H), 7.50–7.95 (7H, 2-naphthyl). ESI-MS: 407.4 (M + H).

(*R*)-2-NMA ester of (*S*)-3-hydroxy-β-ionone (6b). Separated from a reaction of racemic 3-hydroxy-β-ionone with (*R*)-2-NMA, using a chiral column (Chiracel OD), 1 : 99 propan-2-ol–hexane, $\lambda = 280$ nm. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ_H 1.09 (3H, s, 1-Me), 1.13 (3H, s, 1-Me), 1.62 (1H, t, $J = 12$ Hz, 2-Hax), 1.64 (3H, s, 5-Me), 1.81 (1H, ddd, $J = 12$, 3, 2 Hz, 2-Heq), 1.97 (1H, dd, $J = 18$, 9 Hz, 4-Hax), 2.27 (3H, s, 9-Me), 2.35 (1H, dd,

J = 18, 6 Hz, 4-Heq), 3.46 (3H, s, O-Me), 4.91 (1H, s, H-NMA), 5.11 (1H, m, 3-H), 6.05 (1H, d, J = 16 Hz, 8-H), 7.14 (1H, d, J = 16 Hz, 7-H), 7.50–7.92 (7H, 2-naphthyl).

(*R*)-9-ATMA ester of (*R*)-3-hydroxy-β-ionone (7a). Separated from a reaction of racemic 3-hydroxy-β-ionone with (*R*)-9-ATMA, using a chiral column (Chiracel OD), 1 : 99 propan-2-ol–hexane, λ = 280 nm. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ_H 0.74 (3H, s, 1-Me), 0.98 (3H, s, 1-Me), 1.18 (1H, dd, J = 12, 11 Hz, 2-Hax), 1.41 (1H, ddd, J = 12, 3, 1.7 Hz, 2-Heq), 1.68 (3H, s, 5-Me), 2.09 (1H, dd, J = 18, 9 Hz, 4-Hax), 2.25 (3H, s, 9-Me), 2.47 (1H, dd, J = 17, 5 Hz, 4-Heq), 3.46 (3H, s, O-Me), 5.10 (1H, m, 3-H), 5.99 (1H, d, J = 16 Hz, 8-H), 6.26 (1H, s, H-ATMA), 7.07 (1H, d, J = 16 Hz, 7-H), 7.42–8.62 (9H, 9-anthryl).

(*R*)-9-ATMA ester of (*S*)-3-hydroxy-β-ionone (7b). Separated from a reaction of racemic 3-hydroxy-β-ionone with (*R*)-9-ATMA, using a chiral column (Chiracel OD), 1 : 99 propan-2-ol–hexane, λ = 280 nm. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ_H 0.81 (3H, s, 1-Me), 1.03 (3H, s, 1-Me), 1.39 (3H, s, 5-Me), 1.45 (1H, dd, J = 12, 11 Hz, 2-Hax), 1.60 (1H, dd, J = 18, 8 Hz, 4-Hax), 1.70 (1H, ddd, J = 12, 3, 1.7 Hz, 2-Heq), 2.10 (1H, dd, J = 18, 5 Hz, 4-Heq), 2.24 (3H, s, 9-Me), 3.44 (3H, s, O-Me), 5.09 (1H, m, 3-H), 5.83 (1H, d, J = 16 Hz, 8-H), 6.26 (1H, s, H-ATMA), 6.98 (1H, d, J = 16 Hz, 7-H), 7.42–8.55 (9H, 9-anthryl).

(*R*)-MPA ester of (3*R*,3*R*′)-zeaxanthin (8a). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ_H 1.01 (6H, s, 1-Me), 1.05 (6H, s, 1-Me), 1.51 (2H, t, J = 12 Hz, 2-Hax), 1.65 (2H, m, 2-Heq), 1.70 (6H, s, 5-Me), 1.95 (6H, s), 1.97 (6H, s), 2.14 (2H, dd, J = 17, 8 Hz, 4-Hax), 2.45 (2H, dd, J = 17, 5 Hz, 4-Heq), 3.43 (6H, s, O-Me), 4.75 (2H, s, H-MPA), 5.11 (2H, m, 3-H), 6.08 (4H), 6.15 (2H), 6.25 (2H), 6.35 (2H), 6.63 (4H), 7.33–7.47 (10H, phenyl). ESI-MS: 865.4 (M + H).

(*S*)-MPA ester of (3*R*,3*R*′)-zeaxanthin (8b). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ_H 1.06 (6H, s, 1-Me), 1.09 (6H, s, 1-Me), 1.61 (2H, t, J = 12 Hz, 2-Hax), 1.65 (6H, s, 5-Me), 1.79 (2H, m, 2-Heq), 1.95 (6H, s), 1.96 (2H, 4-Hax), 1.97 (6H, s), 2.31 (2H, dd, J = 17, 5 Hz, 4-Heq), 3.43 (6H, s, O-Me), 4.75 (2H, s, H-MPA), 5.12 (2H, m, 3-H), 6.08 (4H), 6.15 (2H), 6.25 (2H), 6.35 (2H), 6.63 (4H), 7.33–7.46 (10H, phenyl).

(*R*)-MPA ester of (all-*E*)-(3*R*)-3-hydroxy-7,8-didehydro-8′-apo-β-caroten-8′-al (9a). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ_H 1.13 (3H, s, 1-Me), 1.13 (3H, s, 1-Me), 1.49 (1H, t, J = 12 Hz, 2-Hax), 1.71 (1H, m, 2-Heq), 1.90 (6H, s, 5-Me), 1.98 (3H, s), 2.01 (6H, s), 2.16 (1H, dd, J = 18, 9 Hz, 4-Hax), 2.50 (1H, dd, J = 17, 5 Hz, 4-Heq), 3.42 (3H, s, O-Me), 4.74 (1H, s, H-MPA), 5.08 (1H, m, 3-H), 6.28 (1H), 6.36 (1H), 6.45 (1H), 6.56 (1H), 6.71 (4H), 6.94 (1H), 7.33–7.45 (5H, phenyl), 9.46 (1H).

(*S*)-MPA ester of (all-*E*)-(3*R*)-3-hydroxy-7,8-didehydro-8′-apo-β-caroten-8′-al (9b). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ_H 1.16 (3H, s, 1-Me), 1.19 (3H, s, 1-Me), 1.59 (1H, t, J = 12 Hz, 2-Hax), 1.86 (1H, m, 2-Heq), 1.84 (3H, s, 5-Me), 1.90 (3H, s), 1.98 (3H, s), 1.99 (1H, dd, J = 18, 9 Hz, 4-Hax), 2.01 (6H, s), 2.36 (1H, dd, J = 17, 5 Hz, 4-Heq), 3.42 (3H, s, O-Me), 4.74 (1H, s, H-MPA), 5.10 (1H, m, 3-H), 6.28 (1H), 6.36 (1H), 6.45 (1H), 6.56 (1H), 6.71 (4H), 6.94 (1H), 7.34–7.45 (5H, phenyl), 9.46 (1H). ESI-MS: 579.7 (M + H).

(*R*)-MPA ester of (all-*E*)-(3*R*)-triophaxanthin (10a). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ_H 1.13 (3H, s, 1-Me), 1.13 (3H, s, 1-Me), 1.49 (1H, t, J = 12 Hz, 2-Hax), 1.71 (1H, ddd, J = 12, 3.5, 2 Hz, 2-Heq), 1.90 (3H, s, 5-Me), 1.94 (3H, s), 1.98 (3H, s), 2.00 (6H, s), 2.16 (1H, dd, J = 18, 9 Hz, 4-Hax), 2.36 (3H, s), 2.50 (1H, dd, J = 17, 5 Hz, 4-Heq), 3.42 (3H, s, O-Me), 4.74 (1H, s, H-MPA), 5.09 (1H, m, 3-H), 6.28 (1H), 6.38 (2H), 6.46 (1H), 6.65 (5H), 7.14 (1H), 7.33–7.45 (5H, phenyl).

(*S*)-MPA ester of (all-*E*)-(3*R*)-triophaxanthin (10b). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ_H 1.19 (3H, s, 1-Me), 1.21 (3H, s, 1-Me), 1.59 (1H, t, J = 12 Hz, 2-Hax), 1.84 (3H, s, 5-Me), 1.85 (1H, m, 2-Heq), 1.94 (3H, s), 1.98 (3H, s), 1.99 (1H, m, 4-Hax), 2.00 (6H, s), 2.36 (1H, dd, J = 17, 5 Hz, 4-Heq), 3.42 (3H, s, O-Me), 4.74 (1H, s, H-MPA), 5.09 (1H, m, 3-H), 6.28 (1H), 6.38 (2H), 6.46 (1H), 6.65 (5H), 7.34–7.45 (5H, phenyl).

(*R*)-MPA ester of (9*Z*)-(3*S*)-apohopkinsiaxanthin (11a). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ_H 1.32 (3H, s, 1-Me), 1.37 (3H, s, 1-Me), 1.93 (1H, m, 2-Hax), 1.95 (6H, s), 1.98 (1H, m, 2-Heq), 2.00 (3H, s), 2.04 (3H, s), 2.05 (3H, s, 5-Me), 3.54 (3H, s, O-Me), 4.93 (1H, s, H-MPA), 5.57 (1H, dd, J = 14, 6 Hz, 3-H), 6.32 (1H), 6.45 (4H), 6.66 (4H), 6.79 (1H), 7.12 (1H), 7.33–7.56 (5H, phenyl). ESI-MS: 607.6 (M + H).

(*S*)-MPA ester of (9*Z*)-(3*S*)-apohopkinsiaxanthin (11b). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ_H 1.32 (3H, s, 1-Me), 1.37 (3H, s, 1-Me), 1.95 (6H, s), 2.00 (3H, s), 2.04 (3H, s), 2.06 (3H, s, 5-Me), 2.07 (1H, m, 2-Hax), 2.13 (1H, m, 2-Heq), 3.55 (3H, s, O-Me), 4.91 (1H, s, H-MPA), 5.56 (1H, dd, J = 14, 6 Hz, 3-H), 6.32 (1H), 6.45 (4H), 6.66 (4H), 6.79 (1H), 7.12 (1H), 7.33–7.56 (5H, phenyl).

(*R*)-MPA ester of (all-*E*)-(3*S*,5*R*,6*R*)-paracentrone (12a). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ_H 1.02 (3H, s, 1-Me), 1.34 (3H, s, 1-Me), 1.34 (1H, m, 2-Hax), 1.34 (3H, s, 5-Me), 1.56 (1H, 4-Hax), 1.79 (3H, s), 1.87 (1H, m, 2-Heq), 1.93 (3H, s), 1.98 (3H, s), 1.99 (3H, s), 2.27 (1H, 4-Heq), 2.36 (3H, s), 3.42 (3H, s, O-Me), 4.74 (1H, s, H-MPA), 5.44 (1H, m, 3-H), 6.05 (1H), 6.11 (1H), 6.28 (1H), 6.35 (1H), 6.39 (1H), 6.67 (5H), 7.15 (1H), 7.34–7.46 (5H, phenyl).

(*S*)-MPA ester of (all-*E*)-(3*S*,5*R*,6*R*)-paracentrone (12b). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ_H 1.06 (3H, s, 1-Me), 1.30 (3H, s, 5-Me), 1.36 (3H, s, 1-Me), 1.44 (1H, 2-Hax), 1.47 (1H, 4-Hax), 1.79 (3H, s), 1.93 (3H, s), 1.98 (3H, s), 1.99 (3H, s), 2.01 (1H, 2-Heq), 2.14 (1H, m, 4-Heq), 2.36 (3H, s), 3.42 (3H, s, O-Me), 4.73 (1H, s, H-MPA), 5.44 (1H, m, 3-H), 6.05 (1H), 6.11 (1H), 6.28 (1H), 6.35 (1H), 6.339 (1H), 6.67 (5H), 7.15 (1H), 7.34–7.45 (5H, phenyl).

(*R*)-MPA ester of (3*R*,3′*R*)-astaxanthin (13a). ^{1}H-NMR (C_6D_6, 500 MHz) δ_H 0.78 (6H, s, 1-Me), 0.82 (6H, s, 1-Me), 1.67 (2H, m, 2-Heq), 1.74 (6H, s), 1.85 (6H, s), 1.90 (2H, m, 2-Hax), 1.99 (6H, s, 5-Me), 3.57 (6H, s, O-Me), 5.05 (2H, s, H-MPA), 5.68 (2H, dd, J = 14, 5 Hz, 3-H), 5.95 (2H), 6.26 (4H), 6.36 (2H), 6.51 (2H), 6.68 (4H), 7.15–7.73 (10H, phenyl).

(*S*)-MPA ester of (3*R*,3′*R*)-astaxanthin (13b). ^{1}H-NMR (C_6D_6, 500 MHz) δ_H 0.76 (6H, s, 1-Me), 0.80 (6H, s, 1-Me), 1.59 (2H, dd, J = 12, 5 Hz, 2-Hax), 1.74 (6H, s), 1.83 (2H, m, 2-Heq), 1.85 (6H, s), 2.02 (6H, s, 5-Me), 3.51 (6H, s, O-Me), 5.05 (2H, s, H-MPA), 5.71 (2H, dd, J = 14, 4 Hz, 3-H), 5.95 (2H), 6.26 (4H), 6.36 (2H), 6.51 (2H), 6.68 (4H), 7.15–7.73 (10H, phenyl). ESI-MS: 893.3 (M + H).

(*R*)-MPA ester of (3*R*,3′*R*)-(all-*E*)-7,8,7′,8′-tetrahydrozeaxanthin-8,8′-dione (14a). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ_H 0.87 (3H, s, 1-Me), 0.91 (6H, s, 1-Me), 1.39 (6H, s, 5-Me), 1.64 (2H, t, J = 12 Hz, 2-Hax), 1.73 (2H, m, 2-Heq), 1.77 (6H, s), 1.98 (6H, s), 2.20 (2H, dd, J = 17, 10 Hz, 4-Hax), 2.33 (2H, dd, J = 17, 6 Hz, 4-Heq), 3.25 (6H, s, O-Me), 3.31 (4H), 4.75 (2H, s, H-MPA), 5.32 (2H, m, 3-H), 6.35 (2H), 6.55 (4H), 6.69 (2H), 7.15–7.64 (10H, phenyl).

(*S*)-MPA ester of (3*R*,3′*R*)-(all-*E*)-7,8,7′,8′-tetrahydrozeaxanthin-8,8′-dione (14b). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ_H 0.94

(6H, s, 1-Me), 0.95 (6H, s, 1-Me), 1.33 (6H, s, 5-Me), 1.72 (2H, t, J = 12 Hz, 2-Hax), 1.77 (6H, s), 1.83 (2H, m, 2-Heq), 1.98 (6H, s), 2.09 (2H, dd, J = 16, 9 Hz, 4-Hax), 2.23 (2H, dd, J = 17, 5 Hz, 4-Heq), 3.21 (6H, s, O-Me), 4.75 (2H, s, H-MPA), 5.30 (2H, m, 3-H), 6.35 (2H), 6.55 (4H), 6.69 (2H), 7.15–7.64 (10H, phenyl). ESI-MS: 896.2 (M + H).

(*R*)-MPA ester of (3*S*,3′*S*)-(all-*E*)-7,8,7′,8′-tetradehydroastaxanthin (15a). ^{1}H-NMR (C_6D_6, 500 MHz) δ_H 0.92 (6H, s, 1-Me), 1.00 (6H, s, 1-Me), 1.62 (2H, dd, J = 13, 5 Hz, 2-Heq), 1.75 (6H, s), 1.76 (2H, t, J = 13 Hz, 2-Hax), 1.81 (6H, s), 2.16 (6H, s, 5-Me), 3.48 (6H, s, O-Me), 5.00 (2H, s, H-MPA), 5.71 (2H, dd, J = 14, 5 Hz, 3-H), 6.27 (2H), 6.34 (2H), 6.37 (2H), 6.62 (2H), 6.69 (2H), 7.15–7.70 (10H, phenyl). ESI-MS: 888.4 (M + H).

(*S*)-MPA ester of (3*S*,3′*S*)-(all-*E*)-7,8,7′,8′-tetradehydroastaxanthin (15b). ^{1}H-NMR (C_6D_6, 500 MHz) δ_H 0.94 (6H, s, 1-Me), 1.03 (6H, s, 1-Me), 1.69 (2H, dd, J = 13 Hz, 2-Heq), 1.75 (6H, s), 1.84 (2H, t, J = 13 Hz, 2-Hax), 1.81 (6H, s), 2.14 (6H, s, 5-Me), 3.53 (6H, s, O-Me), 5.01 (2H, s, H-MPA), 5.62 (2H, dd, J = 14, 5 Hz, 3-H), 6.27 (2H), 6.34 (2H), 6.37 (2H), 6.62 (2H), 6.69 (2H), 7.15–7.77 (10H, phenyl).

(*R*)-MPA ester of lutein (16a). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ_H 0.85 (3H, s, 1′-Me), 0.94 (3H, s, 1′-Me), 1.01 (3H, s, 1-Me), 1.05 (3H, s, 1-Me), 1.45 (1H, dd, J = 14, 5 Hz, 2′-Heq), 1.51 (1H, t, J = 12 Hz, 2-Heq), 1.58 (3H, s, 5′-Me), 1.65 (1H, m, 2-Hax), 1.70 (3H, s, 5-Me), 1.84 (1H, dd, J = 14, 6 Hz, 2′-Hax), 1.89 (3H, s), 1.96 (12H), 2.14 (1H, dd, J = 18, 9 Hz, 4-Hax), 2.35 (1H, d, J = 10 Hz, 6′-H), 2.45 (1H, dd, J = 17, 5 Hz, 4-Heq), 3.43 (6H, s, O-Me and O′-Me), 4.75 (1H, s, H-MPA), 4.77 (1H, s, H′-MPA), 5.11 (1H, m, 3-H), 5.34 (1H, m, 4′-H), 5.35 (1H, m, 3′-H), 6.09 (6H), 6.25 (1H), 6.35 (2H), 6.62 (4H), 7.33–7.46 (10H, phenyl). ESI-MS: 863.7 (M + H).

(*S*)-MPA ester of lutein (16b). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ_H 0.77 (3H, s, 1′-Me), 0.77 (3H, s, 1′-Me), 1.06 (3H, s, 1-Me), 1.09 (3H, s, 1-Me), 1.23 (1H, dd, J = 14, 5 Hz, 2′-Heq), 1.61 (1H, t, J = 12 Hz, 2-Heq), 1.65 (6H, s, 5′-Me and 5-Me), 1.71 (1H, dd, J = 14, 6 Hz, 2′-Hax), 1.79 (1H, m, 2-Hax), 1.89 (3H, s), 1.96 (12H), 1.96 (1H, m, 4-Hax), 2.31 (1H, dd, J = 17, 5 Hz, 4-Heq), 2.32 (1H, d, J = 10 Hz, 6′-H), 3.42 and 3.43 (6H, s, O-Me and O′-Me), 4.73 (1H, s, H-MPA), 4.75 (1H, s, H′-MPA), 5.12 (1H, m, 3-H), 5.38 (1H, m, 3′-H), 5.48 (1H, m, 4′-H), 6.09 (6H), 6.25 (1H), 6.35 (2H), 6.62 (4H), 7.33–7.46 (10H, phenyl). ESI-MS: 863.8 (M + H).

Acknowledgements

We gratefully acknowledge support by the National Institute of Health (GM 34509 to KN and NB) and the Jacob and Alice Wallenbergs Foundation (to TA). We also thank Dr H. Mayer, Roche, Basel for samples of **8**, **13** and **15**, Dr G. Saucy, Roche, Nutley, for sample **14** and Professor T. Kusumi, Tokushima University, Tokushima for kindly providing us with 9-anthrylmethoxyacetic acid (**7**).

References

1 F. Mo, *X-Ray Crystallographic Studies*, ed. G. Britton, S. Liaaen-Jensen and H. Pfander, Birkhäuser, Basel, 1995.
2 S. Liaaen-Jensen, *Fortschr. Chem. Org. Naturst.*, 1980, **39**, 123.
3 R. Buchecker and K. Noack, "Circular dichroism", in *Carotenoids. Vol. 1B. Spectroscopy*, ed. G. Britton, S. Liaaen-Jensen and H. Pfander, Birkhäuser, Basel, 1995.
4 D. Kull and H. Pfander, *Carotenoids. Vol. 1A. Isolation and Analysis*, ed. G. Britton, S. Liaaen-Jensen and H. Pfander, Birkhäuser, Basel, 1995.
5 O. Straub, *Key to Carotenoids*, ed. H. Pfander, Birkhäuser, Basel, 1987.
6 N. Harada and K. Nakanishi, *Circular Dichroic Spectroscopy: Exciton Coupling in Organic Stereochemistry*, University Science Books, Mill Valley, CA, 1983.
7 K. Nakanishi and N. Berova, "The Exciton Chirality Method", in *Circular Dichroism—Principles and Applications*, ed. K. Nakanaishi, N. Berova and R. W. Woody, VCH Publishers, Inc., New York, 1994.
8 R. Person, K. Monde, H.-U. Humpf and N. Berova, *Chirality*, 1995, **7**, 128.
9 K. Nakanishi, in *One-Dimensional and Two-Dimensional NMR Spectra by Modern Pulse Techniques*, Mill Balley, CA, 1990.
10 S. Hertzberg and L. Liaaen-Jensen, unpublished results.
11 J. A. Dale and H. S. Mosher, *J. Am. Chem. Soc.*, 1973, **95**, 512.
12 B. M. Trost, J. L. Belletire, S. Godleski, P. G. McDougal, J. M. Balkovec, J. J. Baldwin, M. E. Christy, G. S. Ponticello, S. L. Varga and J. P. Springer, *J. Org. Chem.*, 1986, **51**, 2370.
13 I. Ohtani, T. Kusumi, Y. Kashman and H. Kakisawa, *J. Am. Chem. Soc.*, 1991, **113**, 4092.
14 G. Uray, in *Methods of Organic Synthesis (Houben-Weyl)*, vol. E21a, Georg Thieme Verlag, Stuttgart, 1995, pp. 253–292.
15 S. K. Latypov, J. M. Seco, E. Quinoa and R. Riguera, *J. Org. Chem.*, 1995, **60**, 504.
16 H. Ok, C. Caldwell, D. R. Schroeder, A. K. Singh and K. Nakanishi, *Tetrahedron Lett.*, 1988, **29**, 2275.
17 T. Kusumi, H. Takahashi, P. Xu, T. Fukushima, Y. Asakawa, T. Hashimoto, Y. Kan and Y. Inouye, *Tetrahedron Lett.*, 1994, **35**, 4397.
18 A. Rüttimann and H. Mayer, *Helv. Chim. Acta*, 1989, **63**, 1456.
19 J. A. Haugan, *J. Chem. Soc.*, *Perkin Trans. 1*, 1997, **18**, 2731.
20 J. A. Haugan and S. Liaaen-Jensen, *Acta Chem. Scand.*, 1994, **48**, 899.
21 J. A. Haugan, *Acta Chem. Scand.*, 1997, **51**, 1096.
22 J. H. Haugan, E. Lobkovsky and S. Liaaen-Jensen, *Acta Chem. Scand.*, 1997, **51**, 1201.
23 K. Bernhard, F. Kienzle, H. Mayer and R. K. Müller, *Helv. Chim. Acta*, 1980, **63**, 1473.
24 F. Kienzle and H. Mayer, *Helv. Chim. Acta*, 1978, **61**, 2609.
25 G. Saucy, unpublished results (formerly at Hoffman-La Roche, Nutley, NJ, USA).
26 H. Mayer and A. Rüttimann, *Helv. Chim. Acta*, 1980, **63**, 1451.
27 K. S. Latypov, J. M. Seco, E. Quinoa and R. Riguera, *J. Am. Chem. Soc.*, 1998, **120**, 877.
28 R. Buchecker, P. Hamm and C. H. Eugster, *Chimia*, 1971, **25**, 192.
29 A. G. Andrews, G. Borch and S. Liaaen-Jensen, *Acta Chem. Scand.*, *Ser. B*, 1974, **28**, 139.