Stereochemistry of Formation of the β-Ring of Lycopene: Biosynthesis of (1*R*,1′*R*)-β,β-[16,16,16,16′,16′,16′-$^2H_6$]Carotene from [16,16,16,16′,16′,16′-$^2H_6$]Lycopene in *Flavobacterium R 1560*

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Dedicated to Prof. *Albert Eschenmoser* on the occasion of his 75th birthday

Incubation of deuteriated precursors in cultures of *Flavobacterium* produced specifically deuteriated carotenoids. Analysis of these led to several conclusions: *i*) Lycopene is a direct precursor of β,β-carotene. *ii*) Its terminal Me groups retain their integrity during cyclization: there is a stereospecific folding of the 1,5-diene. The Me(16,16′) groups of lycopene become the Me(16,16′) of β,β-carotene. Consequently, the folding must follow the $C_2(E,E)$ mode. *iii*) Incorporation of deuterium was sufficiently extensive to permit CD measurements on the isolated β,β-carotene, allowing its centers of chirality to be assigned as (1*S*,1′*S*). *iv*) The same chirality resulted from incorporation of [$^2H_3$]mevalonate into zeaxanthin. The syntheses of specifically deuteriated [$^2H_3$]GPP, [$^2H_3$]FPP, and [$^2H_3$]GG are described.

1. Introduction. – Only those carotenoids with cyclic end-groups, such as β,β-carotene, are capable of performing this group's vital functions as supplementary light absorbers and deactivators of electronically excited states of chlorophyll in higher plants. As well as this, only those carotenoids with β-rings also function as precursors of retinoids such as, *e.g.*, vitamin A.

Using different experimental approaches, many groups have been able to show recently that lycopene (**1**) is the direct precursor in the biological cyclization giving rise to β,β-carotene[2]). This work offers further confirmation of that finding, and, in addition, offers innovations in methodology.

It also represents the final conclusions of a long-running project in our group, with important preliminary work done by *Märki et al.* [3] and *Hofer et al.* [3d][4], and confirms findings by *Britton et al.* [5] that establish the stereochemistry of the ring closure to zeaxanthin (**2**; *Scheme 1*). Our results, obtained with novel methodology, are also significant in that *Britton*'s findings had conflicted with some reported by *Bu'Lock* and co-workers [6] (*cf. Sect. 5*).

2. Key Reactions. – The controlled degradation of abietic acid (**3a**) and its deuteriated form **3b** to precursors of (1*R*,1′*R*)-β,β-[16,16,16,16′,16′,16′-$^2H_6$]carotene (**4**)

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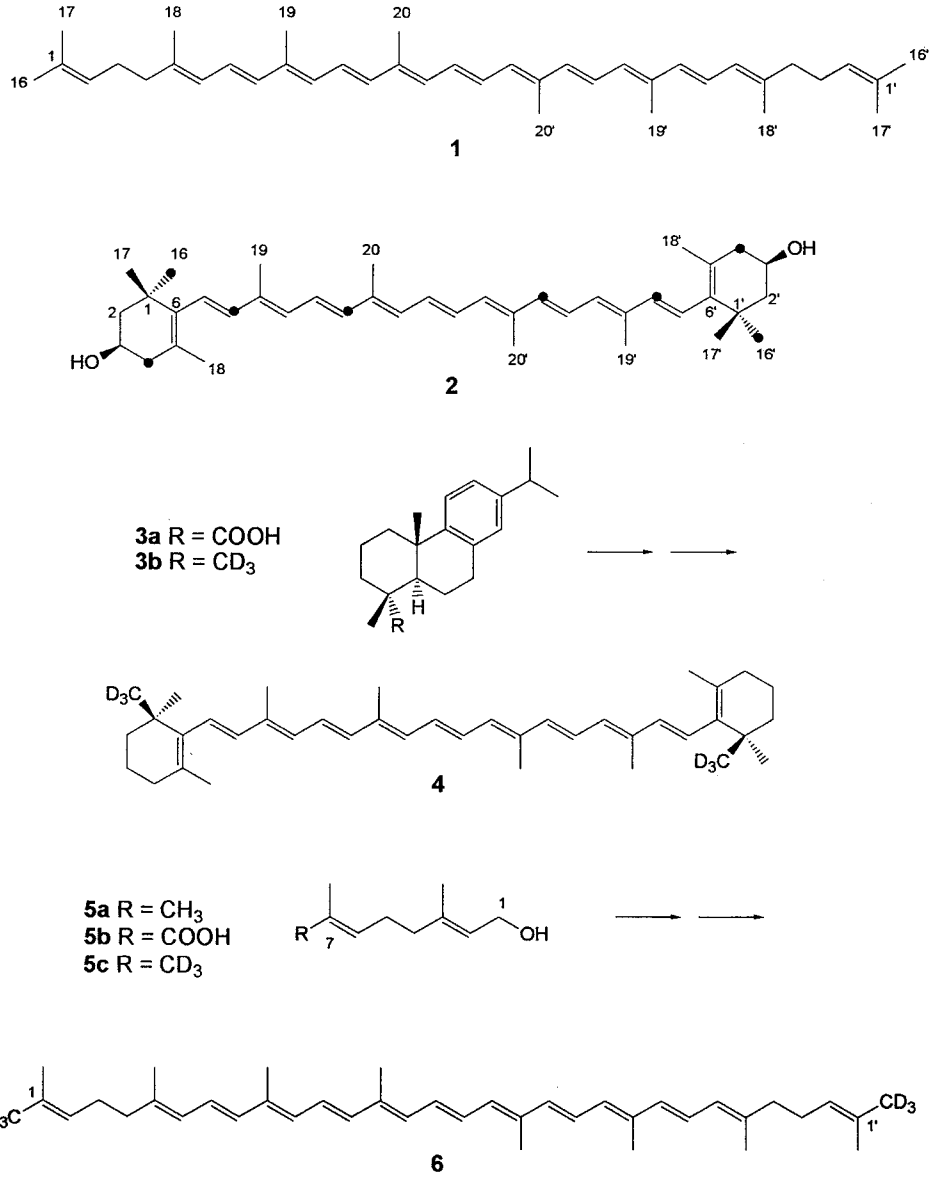
[1]) From the Ph.D. thesis of *S.S.M.* [1]. For external reasons, publication in this journal has not been possible until now. Some fragmentary findings were presented in brief in Chapt. 9 of [2]. This work presents the complete results of the work in Zürich and sets it in its overall context.

[2]) Authoritative review by *Britton* in [2].

Scheme 1



was described in [3]. In this work, labeling has also been used to show that the Me groups at C(1) arising from this, indistinguishable by ^{1}H- and ^{13}C-NMR spectroscopy, produce in the conformational equilibrium an isotope effect sufficiently large as to make it possible to draw reliable conclusions about the chirality of substitution at C(1) and/or C(1′) from low-temperature CD measurements.

Subsequently, we prepared the specifically deuteriated (*E*)-[$^2H_6$]geraniol **5c** and lycopene **6**[3]).

It is unnecessary to stress that, as reference substances for carotenes and carotenoids of biological origin, compound **4** and other isomers not mentioned here [3] were from the outset characterized sufficiently thoroughly that definitive comparisons could be carried out even with the smallest quantities.

Using compound **6**, we carried out numerous incorporation experiments; these, however, did not provide any usable results. Among them, we should mention the wicking technique in yellow roses[4]); injections into ripening rose hips, fruiting bodies of *Inocybe* species, and fruits of *Taxus baccata*; incubations with acetone powders from tomatoes [8]; cell-free enzyme preparations from spinach chloroplasts [8] and those from 'french beans' (*Phaseolus vulgaris* according to [9], *Phycomyces vulgaris* according to [10]), *etc*. However, in each case[5]), we were unsuccessful in identifying any incorporation (detectable by HPLC, MS, FTIR, or CD) of **4** or its enantiomer. On *Britton's* advice, we finally succeeded with an incorporation of **6** in cultures of *Flavobacterium mutante R 1560*[6]) (*Sect. 3.1* and *3.2*).

3. Results. – 3.1. *Biosynthesis of (1S,1′S,3R,3′R)-[17,17′,18,18′,19,19′,20,20′-$^2H_{24}$]Zeaxanthin.* – For our labeled precursor, we used (±)-[3′,3′,3′-$^2H_6$]mevalonolactone (**14**) or [$^2H_3$]mevalonic acid ([$^2H_3$]MVA), following the procedure of *Chakraborty et al*. [11], with the modifications necessary for labeling (*Scheme 2*).

The bacterial cell mass (10000 g) obtained from centrifugation of the cultured aforementioned *Flavobacterium* was incubated in 0.1M *Tris*·HCl buffer, pH 7.0, with lysozyme, $MnSO_4$, $MgSO_4$, adenosine triphosphate (ATP), and the potassium salt of **14** for 24 h at room temperature, under light and with shaking. Subsequently, the carotenoids formed were extracted, saponified, and concentrated by phase separation. The crude zeaxanthin obtained from the hypophase was purified by column chromatography (CC) on $ZnCO_3$/*Celite*.

The FT-IR spectrum showed the presence of deuterium in the molecule. As the signals of Me(16,16′,17,17′) coincide in the ^{1}H-NMR spectrum, the mixture was acetylated to allow discrimination of the terminal Me groups [12]. From this spectrum, it was possible to establish that the intensities of signals of Me(17,17′,18,18′,19,19′,20,20′) were significantly reduced in comparison with those of Me(16,16′). From this reduction, we inferred *ca*. 10% incorporation of deuterium into **15**.

The ^{2}H-NMR spectrum showed three signals in a ratio of 2:1:1 (*Fig. 1*). The signal at 1.07 ppm corresponds to CD_3(17,17′), that at 1.72 ppm to CD_3(18,18′), and the most intense, at 1.96 ppm, to CD_3(19,19′,20,20′). A CD spectrum of **15b**, recorded at room temperature (*Fig. 2*), agrees completely with that of unlabeled (3*R*,3′*R*)-zeaxanthin [13]. This finding confirms that the ring closure leaves intact the identity of the terminal

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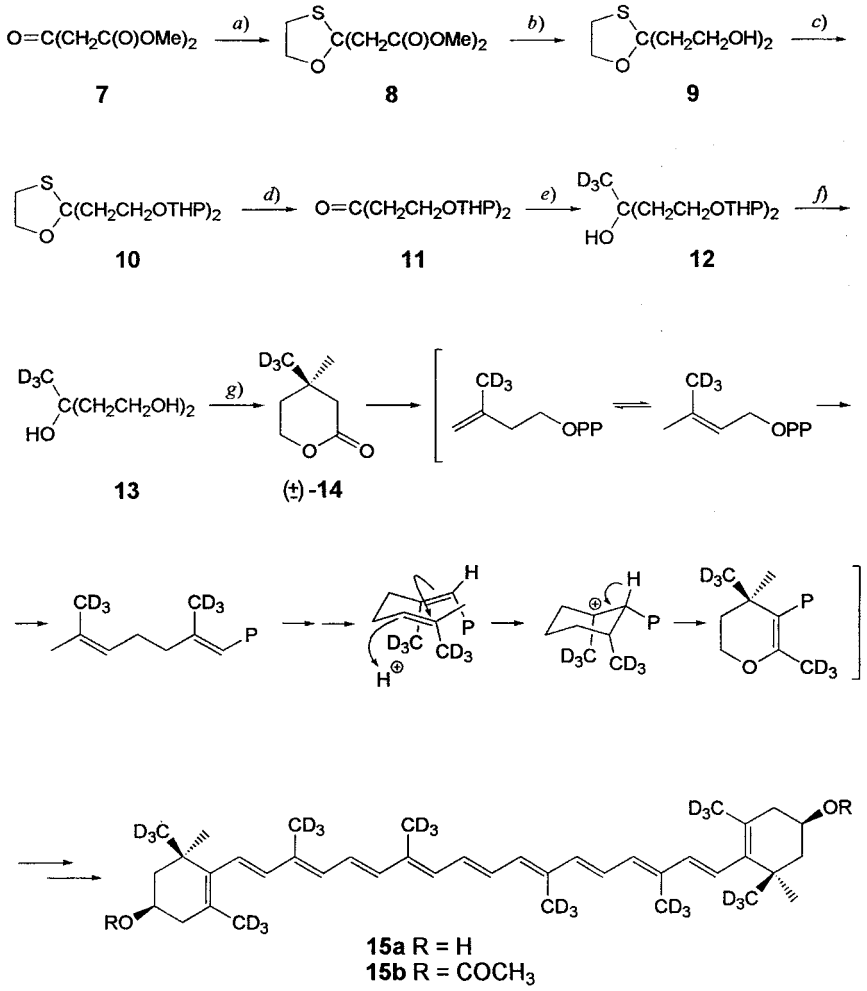
3) The Me groups at C(7), or C(1) and C(1′) of **5** and **6** may be distinguished and assigned by NMR spectroscopy; in **5a** and **5c**, they can also be distinguished by GC [4].

4) For a review of carotenoids in rose flowers, see [7].

5) Incorporation experiments were carried out by *S. S. M*. and by Dipl. Biochem. *A. Eisenring*.

6) *Flavobacterium R 1560* is a gram-negative organism, obtained by *F. Hoffmann LaRoche microbiologists via* mutation of the wild type. Its main carotenoid is zeaxanthin.

Scheme 2



a) $HOCH_2CH_2SH$, $BF_3 \cdot OEt_2$. *b*) $LiAlH_4$, THF. *c*) 3,4-Dihydro-2*H*-pyran, Pyridinium toluene-4-sulfonate. *d*) $HgCl_2$, $CaCO_3$, H_2O, MeCN. *e*) CD_3MgI. *f*) TsOH, H_2O. *g*) Pyridinium chlorochromate, CH_2Cl_2.

Me groups. Our findings complement the experiments carried out on the same organism with [2-^{13}C]MVA by *Britton et al.* [5]; (see **2** in *Scheme 1* and *Sect. 5*).

We assume that the incorporation of **14** (or its K salt) into **15** proceeds through the intermediates shown in brackets (*Scheme 2*). If (all-*E*)-lycopene serves as precursor and the acid-catalyzed cyclization proceeds by the concerted *re(2),si(2),si(6)* electron migration, then this mode of cyclization corresponds to the $C_2(E,E)$ scheme systemized by us [14].

3.2. *Direct Incorporation of (all-*E*)-[16,16,16,16′,16′,16′-$^2H_6$]Lycopene into (all-*E*,1*R*,1′*R*)-β,β-[16,16,16,16′,16′,16′-D_6]Carotene* (**17**; *Scheme 3*). – The main carotenoid of *Flavobacterium R 1560* is (all-*E*,3*R*,3′*R*)-zeaxanthin. β,β-Carotene is also formed in

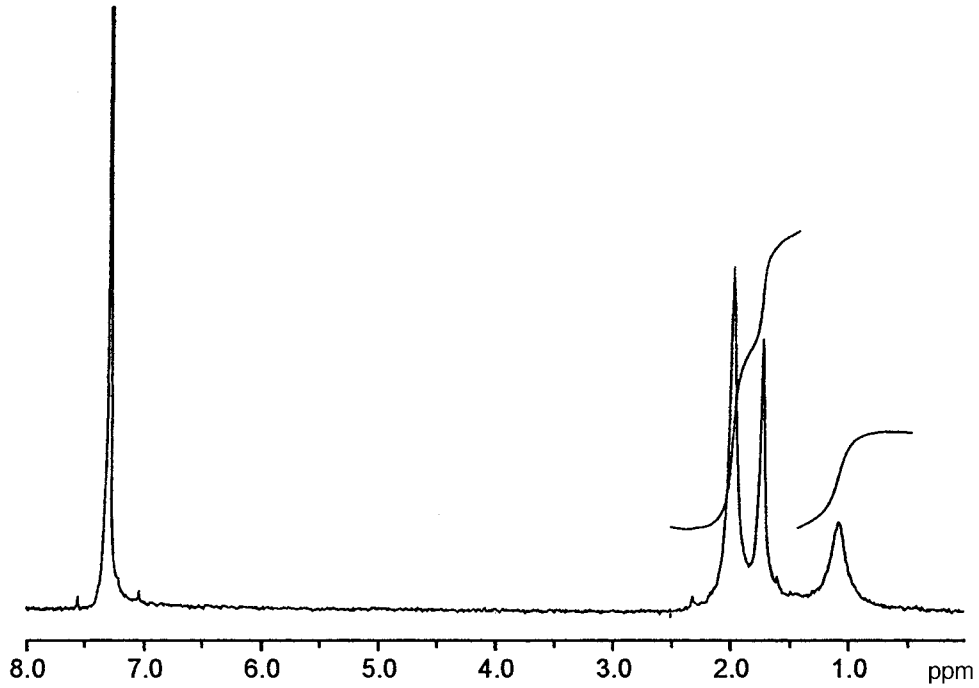


Fig. 1. *^{2}H-NMR Spectrum of biosynthetic di-*O*-acetylzeaxanthin* (**15b**)

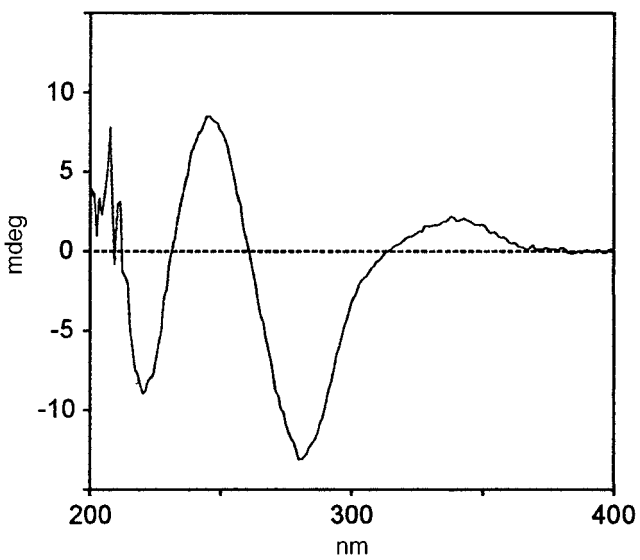
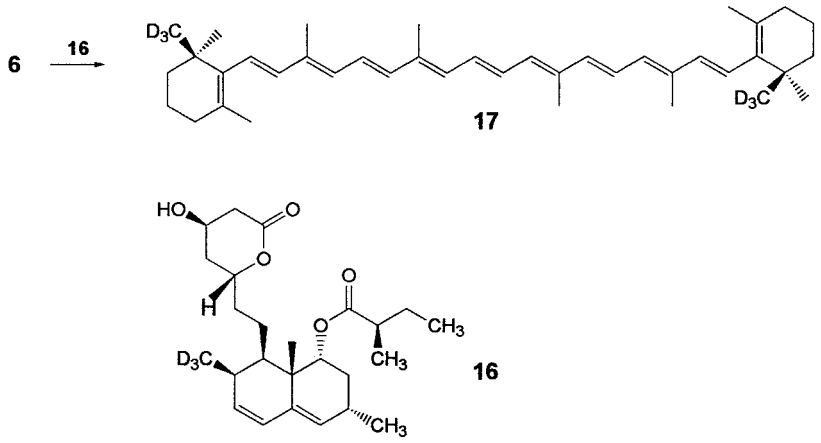


Fig. 2. *Qualitative CD spectrum of di-*O*-acetylzeaxanthin* (**15b**) *at room temperature in Et_2O/isopentane/EtOH 5:5:2* (EPA)

Scheme 3



small amounts, however [15]. There is wide agreement among other authors that this is the precursor of the monohydroxylated product kryptoxanthin, and this in turn of zeaxanthin.

To achieve a sufficiently high degree of labeling in the precursor, it is necessary to reduce *endogenous* production of *β,β*-carotene. To this end, we added *mevinolin* (**16**)[7]) to the culture solution, together with the labeled lycopene (**6**). In addition, to facilitate incorporation of the hydrophobic lycopene, we performed these experiments using a *cell-free* system: a culture of *Flavobacterium* was homogenized in a '*French* press' in three stages (at 10000 psi (= 689 bar) in each case, with some deoxyribonuclease-I (DNAase I) also added at each stage). In this way, the greater portion of cells was lysed. The homogenate was separated from the remaining intact cells by centrifugation at 6000 × G. The clear supernatant obtained contained *ca.* 165 mg/ml protein.

3.3. *Incubations.* Each of 400 individual samples containing 3 ml of supernatant, with 20 µl of **6** in 0.1 ml of EtOH, 10 mg of **16**, and 1 ml of 0.1M *Tris* · HCl buffer, pH 7.0, added, was shaken at 30° under light. In total, 8 mg of **6** were consumed in this way. After 24 h, incubation was terminated by addition of a small quantity of MeOH.

The carotenoids formed were extracted with Et_2O, saponified, and separated into hypophasic and epiphasic fractions; carbohydrates were then separated by CC on aluminium oxide and silica gel. Finally, 5 mg of *β,β*-carotene were obtained, still containing (*Z*)-isomers. Separation of these was achieved by semipreparative HPLC. The HPLC profile and the ^{2}H-NMR spectrum of **17** are shown in *Figs. 3* and *4*, respectively; the qualitative CD spectrum of **17** is shown in *Fig. 5* along with the analogous spectrum of **4** for comparison; in *Fig. 6* is demonstrated how the labeled molecule **17** can easily be detected by FTIR even in a mixture containing unlabeled *β,β*-carotene in a ratio of 1:99. The data show that we were able to obtain a largely pure mixture of **17** and unlabeled *β,β*-carotene.

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[7]) Mevinolin (**16**), isolated and structurally interpreted by *Alberts et al.* [16], is a potent inhibitor of HMG coenzyme reductase (HMG = 3-(hydroxymethyl)glutaric acid), which, for its part, is a precursor of mevalonic acid (MVA) or its lactone **14**.

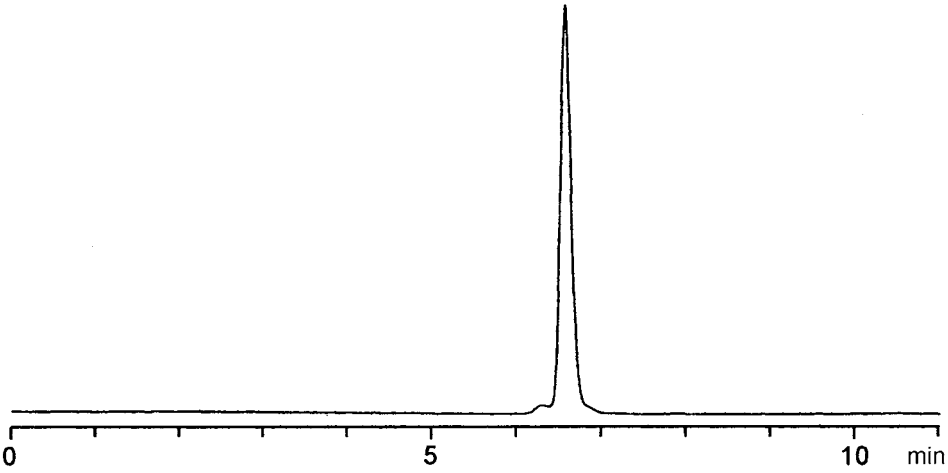


Fig. 3. *HPLC of biosynthetic (1S,1′S)-[16,16,16,16′,16′,16′-$^2H_6$]-β,β-carotene* (**17**) (on *Spherisorb S5-NH_2*, with hexane/*N*-ethyldiisopropylamine 99.9 : 0.1; flow 0.5 ml; detection 445 nm)

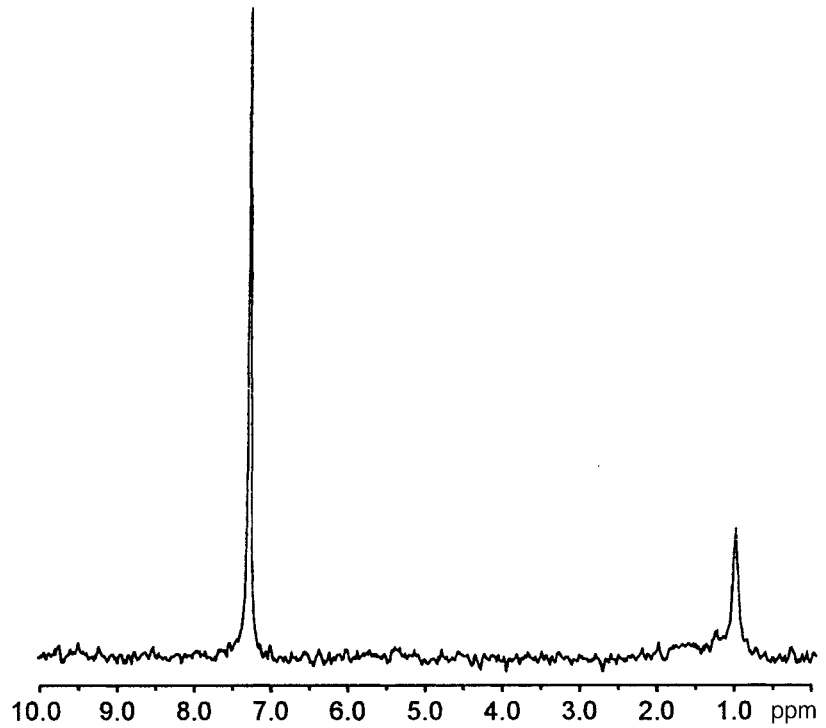


Fig. 4. *^2H-NMR Spectrum of biosynthetic (1S,1′S)-[16,16,16,16′,16′,16′-$^2H_6$]-β,β-carotene* (**17**) *in $CHCl_3$*

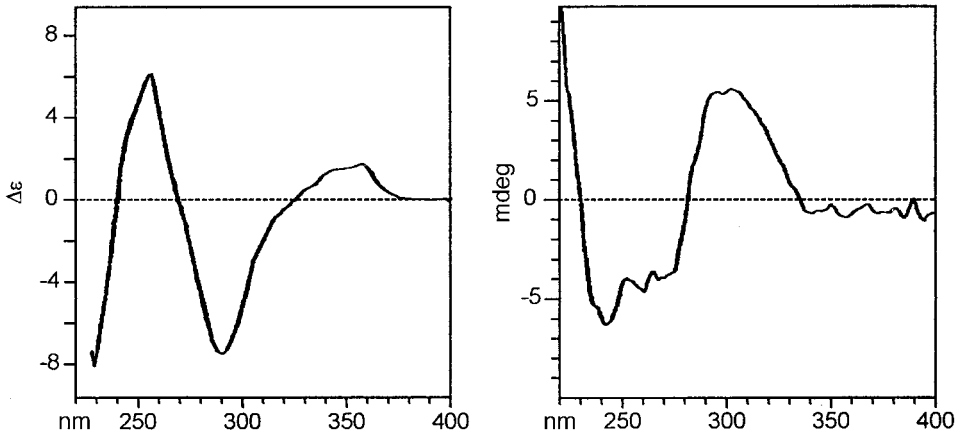


Fig. 5. *Quantitative CD spectrum of semisynthetic (1*R,*1′*R*)-[16,16,16,16′,16′,16′-$^2H_6$]-β,β*-carotene (**4**) *at* −180° [3] (left) *and of biosynthetic (1*S,*1′*S*)-[16,16,16,16′,16′,16′-$^2H_6$]-β,β-carotene* (**17**) (right). Qualitative in EPA at −180°.

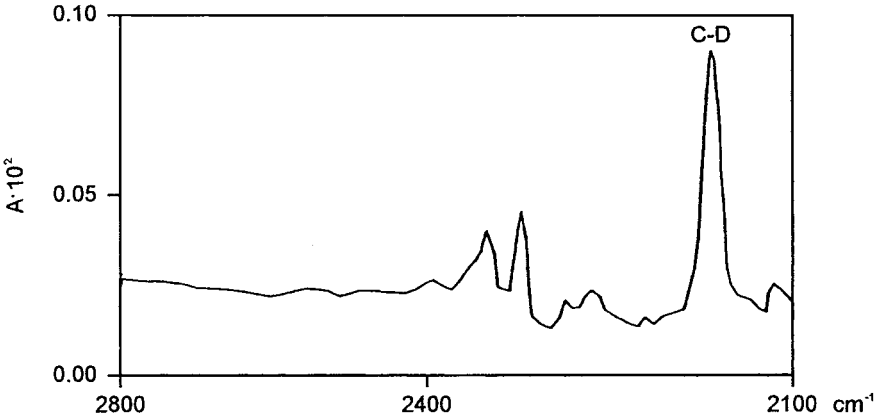


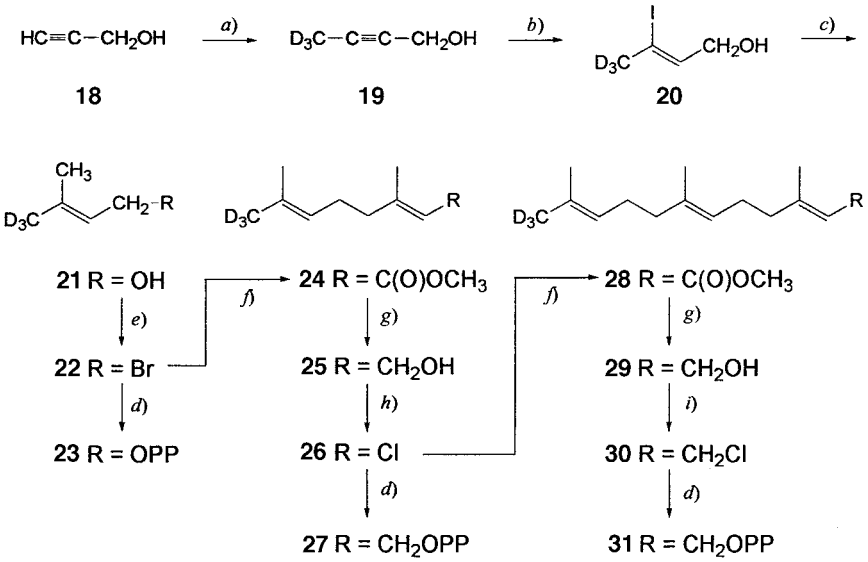
Fig. 6. *IR Difference spectrum between unlabeled β,β-carotene and biosynthetic* **17** (99 : 1 in $CHCl_3$)

The CD curve shows that the labeled *β,β*-carotene is the enantiomer of **4**. Comparing the CD spectrum with the corresponding VIS spectrum, we concluded that the rate of incorporation was 5–10%. Hence, despite the mevinolin (**16**), an appreciably high degree of dilution by endogenous *β,β*-carotene had taken place. That dilution by enantiomeric **4** might also have occurred, however, we were unable to rule out. However, we believe that we can discount this possibility on the grounds of the isolation of **15** in the first experiment.

3.4. *Synthesis of Specifically Deuterated Terpenoids.* To determine the absolute configuration of a biosynthetically obtained cyclized carotene by CD spectroscopy, a relatively high degree of incorporation is essential. Since, over a long period, we had been unable to achieve such a level, the following deuterated terpenoids and their diphosphoric acid esters were prepared as possible precursors.

[$^2H_3$]Dimethylallyl Alcohol ([$^2H_3$]DMAA; 3-methyl[4,4,4-$^2H_3$]but-2-en-1-ol; **21**) and its *pyrophosphate* (DMAPP, **23**) were prepared as outlined in *Scheme 4*. According to the procedure of *Corey et al.* [17], propynol (**18**) was deprotonated, alkylated with CD_3I, and stereoselectively converted to **20** with $LiAlH_4$/NaOMe and I_2. Treatment with Me_2CuLi produced the C_5 alcohol **21.** [$^2H_3$]DMAPP **23** was then prepared from this by a procedure of *Poulter* and co-workers [18], *via* the bromide **22**. The structure of **23** was confirmed by its ^{1}H- and ^{32}P-NMR spectra. The signal of the (*E*)-Me group, prominent in the unlabeled reference compound, was completely absent in **23**. In comparison with the free alcohol, H–C(1) is shifted by *ca.* 0.3 ppm. The chemical shifts of P(α) and P(β) matched literature values.

Scheme 4



a) $LiNH_2$, NH_3, CD_3I. *b*) $LiAlH_4$, NaOMe, THF, I_2. *c*) Me_2CuLi. *d*) Tris(tetrabutylammonium)hydrogen diphosphate. *e*) PBr_3, Et_2O. *f*) 1. $MeC(O)CH_2C(O)OMe$, NaH, THF; 2. BuLi, Hexane, $ClPO(OEt)_2$; 3. Me_2CuLi. *g*) DIBAH, Hexane. *h*) CCl_4, Ph_3P. *i*) *N*-Chlorosuccinimide, MeSMe.

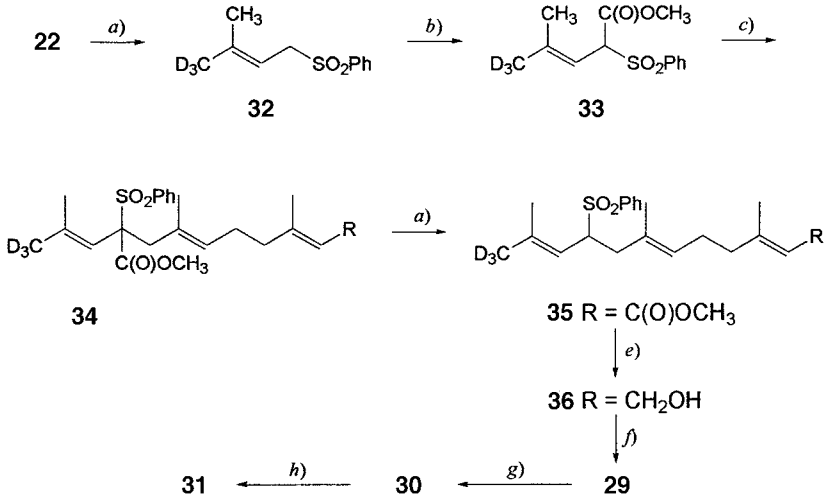
[$^2H_3$]Geraniol ([$^2H_3$]GA, **25**) *and [$^2H_3$]GPP* (**27**). For the starting material, we used the alcohol **21** and its bromide **22** (*Scheme 4*). Extension by five C-atoms was accomplished in a one-pot procedure by the method of *Weiler* and co-workers [19]. The dianion of ethyl acetoacetate was regioselectively alkylated in the γ-position with **22**, the intermediate enolate then converted to the enol phosphate with chlorodiethylphosphoric acid, and this was finally substituted with a Me group from Me_2CuLi. The C(2)=C(3) bond retains its (*E*)-configuration in this reaction sequence.

We isolated the monoterpene ester **24** in 73% yield. After reduction to geraniol **25** and its conversion to the chloride **26** and subsequent pyrophosphoric acid esterification according to [18], we obtained [$^2H_3$]GPP (**27**) in good yield. Its spectroscopic data agreed with those of a reference compound prepared by a different route [4].

[$^2H_3$]Farnesol (**29**, [$^2H_3$]FA) *and [$^2H_3$]FPP* (**31**) were prepared in two different ways (*Schemes 4* and *5*). First, geraniol **25** was converted to the chloride **26** with Ph_3P/CCl_4 [20], and this was then extended by the already mentioned *Weiler* procedure [19]. In contrast to the preparation of **24**, it was necessary in this instance to isolate the intermediate enol phosphate, as the Me_2CuLi treatment proceeded only incompletely otherwise. Reduction of **28** to [$^2H_3$]farnesol (**29**) and pyrophosphoric acid esterification of the chloride **30** to give **31** were carried out as described for [$^2H_3$]geraniol (**25**).

For the second route, the chloride **22** was converted to the sulfone **32** with $NaSO_2Ph$. We converted the anion of this to the more strongly acidic **33** with methyl chloroformate. This was extended by ten C-atoms with the palladium complex of methyl geranate according to the procedure of *Trost et al.* [21].

Scheme 5



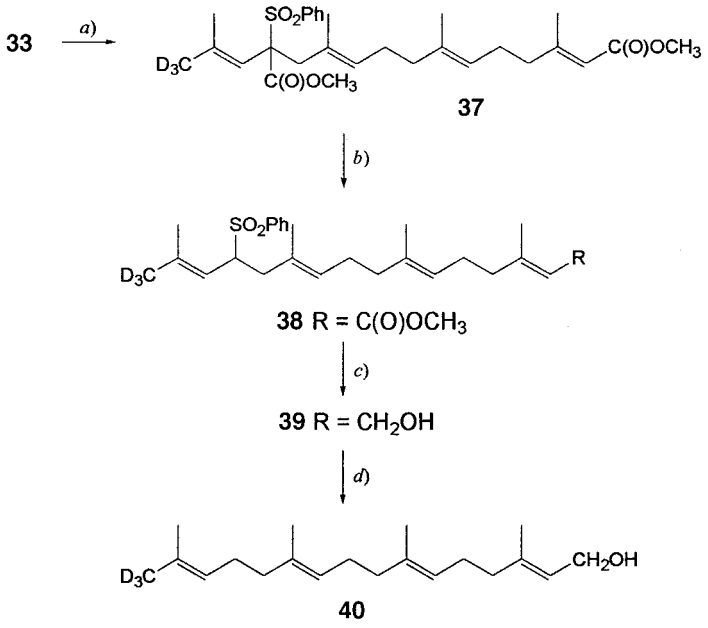
a) $NaSO_2Ph$. *b*) BuLi, ClCOMe. *c*) π-Pd complex of methyl geranate, Ph_3P, NaH, THF. *d*) 4-Aminothiophenol, Cs_2CO_3. *e*) DIBAH, Hexane. *f*) Li, $EtNH_2$. *g*) *N*-Chlorosuccinimide, MeSMe. *h*) Tris(tetrabutylammonium) hydrogen diphosphate.

As expected, only the Me signal at 1.56 ppm appeared in the NMR spectrum of sulfone **34**; hence, the reaction was stereospecific. After decarboxylation of **34** with LiI/NaCN [21], we were able to isolate only 44% of **35**. We obtained double that yield, however, by using the method of *Keinan* and *Eren* [22]. After reduction of ester **35** to **36** and subsequent desulfonation according to [22], we once more obtained **29**, which had spectra identical to those obtained from the sample prepared by the first route. Apart from the lack of the terminal (*E*)-Me group, its identity with non-deuterated farnesol was also confirmed. Conversion of **36** to chloride **30** and diphosphorylation [18] finally supplied **31** once more.

[$^2H_3$]Geranylgeraniol ([$^2H_3$]GGA; *Scheme 6*). The extension technique employed in the second route to [$^2H_3$]farnesol **29** (*Scheme 5*) was used, but with **33** as starting material. Spectroscopically, the isolated [$^2H_3$]GGA was identical to non-deuterated geranylgeraniol [21], apart from the lack of any signal from the terminal (*E*)-Me group.

In the ^{2}H-NMR spectrum, the signal of the CD_3 group lies at 1.65 ppm. The planned diphosphorylation encountered difficulties and so was not pursued further.

Scheme 6



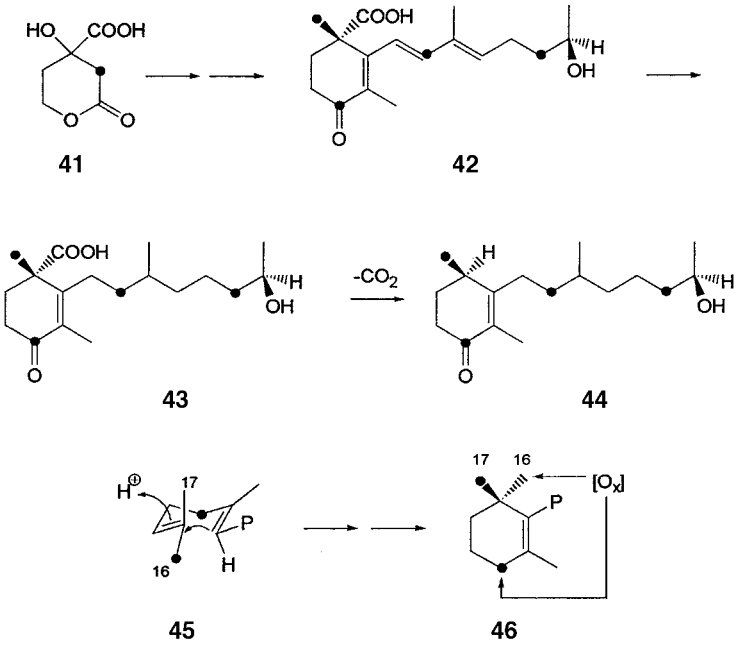
a) 1. $PdCl_2$, $CuCl_2$, methyl farnesate, 2. Ph_3P, NaH, THF. *b*) 4-Aminothiophenol, Cs_2CO_3. *c*) DIBAH, Hexane. *d*) Li, $EtNH_2$.

4. Discussion. – The theory, set out quite some years ago, that lycopene is the most important precursor of carotenes with cyclic end groups (summarized by *Goodwin* in [23]) has now been conclusively proven by our experiments.

As regards the problem of the stereochemisty of folding of the 1,5-diene (in lycopene) and the chirality about C(1) in the β-end group, two groups have already provided an answer, but with mutually contradictory results. To summarize briefly: *Bu'Lock et al.* [6] isolated trisporic acid C from a *Blakslea trispora* culture, and the structure **42** was assigned to it on the basis of extensively interpreted CD spectra and of its further transformation (*Scheme 7*). Since the fungus also produced β,β-carotene as well, the authors viewed **42** as a degradation product of β,β-carotene.

After addition of [2-^{14}C]mevalolactone (**41**) to the culture medium, it proved possible to isolate radiolabeled trisporic acid C, with an incorporation rate of 0.6%. The decisive experiment to explain the distribution of radioactivity in the prochiral Me groups at C(1) was carried out by selective hydrogenation of the side-chain C=C bond, followed by thermal decarboxylation of the vinylogous β-keto carboxylic acid **43** to **44**. The captured CO_2 showed an activity of 1.4% of the total activity of **43**, against 99.3% for **44**. From this, it was possible to infer a sterically specific folding of the 1,5-diene in chair-like manner, as anticipated in formula **45**. By our nomenclature, this would correspond to folding in C_1-(*E*,*E*) mode [14] with *si(2)*,*re(1)*,*si(6)* concerted electron

Scheme 7



migration. After oxidation of the β,β-carotene $Me_{pro\text{-}S}$ group (= Me(16)) to a C=O group, introduction of the oxo group at C(4) and degradation of the side chain at C(1), the result is trisporic acid C (**42**)[8]).

Using a completely different experimental approach, *Britton et al.* [5] later found a quite different result with *Flavobacterium R 1560*. Incorporation of [2-^{13}C]mevalolactone in the presence of the cyclase inhibitor nicotine produced a labeled lycopene in which, according to NMR analysis, enhanced labeling had occurred at Me(16) and Me(16′), but not at Me(17) and Me(17′). Nicotine-free cultures provided zeaxanthin (**2**), likewise with labeling at Me(16) and Me(16′). From these results, *Britton et al.* inferred a folding of the lycopene (as the zeaxanthin precursor) by the C_2-(*E*,*E*) mode (*Scheme 2*). Further incorporation experiments made it possible to confirm and enlarge on these results [24].

Our findings (*Sects. 3.1* and *3.2*) supplement the conclusions of *Britton et al.* [5]. To interpret the apparently contradictory findings of *Bu'Lock* and co-workers [6], we tend to favor the assumption of an *organism-specific* cyclization. It is true that, to date, only a few confirmed instances of enantiomeric folding of the 1,5-diene are known in carotenoid biogenesis, quite in contrast to the many examples in the diterpenoid series.

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[8]) The $Me_{pro\text{-}S}$ group in the β-ring is given the locant 16 according to carotenoid nomenclature. Hence, it corresponds biogenically to the same numbering in the precursor lycopene, provided that its folding follows the C_2-(*E*,*E*) mode (see *Schemes 2* and *3*). However, an inversion in the numbering in fact occurred in the C_2-(*E*,*E*) mode (see *Scheme 7*).

They mostly involve the variable sense of chirality at C(6) in carotenes with an ε end group[9]).

Although the (6*R*,6′*R*)-ε,ε-carotene is found in tomatoes [25], the (6*S*,6′*S*)-enantiomer is found in algae [26]. We have postulated a (5*Z*)-lycopene precursor as a prerequisite for the formation of an ε- rather than a β-ring [27].

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Experimental Part

General. See [28]. Solvents used for reactions were of anal. quality and were dried carefully before use. For particulars of biological procedures see [1], p. 62–65 and 83–84.

1. *Incorporation of [3′,3′,3′-$^2H_3$]MVA* ((±)-**14**). The culture of bacteria in liquid medium was carried out in modified 2-l *Erlenmeyer* flasks, each containing 500 ml of culture medium in an orbital incubator at 160 rpm at r.t., under light, over 30 h. The ensuing sedimentation of bacteria was accomplished at 10000 × g, followed by 3-fold washing with 0.1M *Tris* · HCl buffer, pH 7.0. The bacterial mass from 4 l of culture was suspended in 0.1M *Tris* · HCl buffer (320 ml) and divided into 40-ml portions in 8 *Erlenmeyer* flasks. To each incubation experiment was added the K salt of (±)-**14** (40 mg), hen egg albumin lysozyme (60 mg), $MnSO_4 \cdot 4\,H_2O$ (22 mg), $MgSO_4 \cdot 7\,H_2O$ (75 mg), and ATP (100 mg) dissolved in *Tris* · HCl buffer (10 ml), and the incubations were carried out as for the bacterial cultures over 24 h.

2. *Isolation of Zeaxanthin* (**15a**). The combined products were filtered over *Celite*, then extracted with acetone until colorless, the filtrate diluted with Et_2O and thoroughly washed with H_2O. After drying and removal of solvent, saponification was performed with 10% KOH in MeOH. The usual extraction was performed, followed by phase separation between 95% MeOH and hexane. CC of hypophasic carotenoids on silica gel with acetone/hexane 4:1 gave 40 mg of zeaxanthin, which was acetylated with Ac_2O/pyridine. Purification of the acetate was performed by prep. HPLC. CD: see *Fig. 2*. FT-IR (*Fig. 6*; $CHCl_3$; difference spectrum): 2241 (sh), 2214*s*, 2173*w*, 2106*w*, 2063*s*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.074 (*s*, Me(17,17′)); 1.105 (*s*, Me(16,16′)); 1.583 (*t*, ^3J = 12, H–C(2,2′)); 1.721 (*s*, Me(18,18′)); 1.775 (*dd*, ^3J = 12, H–C(2,2′)); 1.966, 1.972 (2*s*, Me(19,19′,20,20′)); 2.049 (*s*, 2 Ac); 2.108 (*dd*, H–C(4,4′)); 2.439 (*dd*, H–C(4,4′)); 5.059 (*m*, H–C(3,3′)); 6.101 (*m*, H–C(7,7′)); 6.114 (*m*, H–C(8,8′)); 6.160 (*m*, H–C(10,10′)); 6.257 (*m*, H–C(14,14′)); 6.365 (*m*, H–C(12,12′)); 6.607–6.995 (*m*, H–C(11,11′,15,15′)). ^{2}H-NMR (*Fig. 1*; 61.4 MHz, $CDCl_3$): 1.072 (*s*, CD_3(17,17′)); 1.720 (*s*, CD_3(18,18′)); 1.963 (*s*, CD_3(19,19′,20,20′)).

3. *Preparation of a Cell-Free System from* Flavobacterium R 1560. The bacterial mass from 4 l of culture was isolated by centrifugation, washed several times with 0.1M *Tris* · HCl buffer (pH 7), and finally suspended in the same buffer (40 ml). With a '*French* press' (*Aminco*), the cells were homogenized 3 times at 4° and 10000 psi. After each pass, a few mg of DNAase I were added to the soln. Non-homogenized material and residual cell wall and cell membrane were then separated by centrifugation for 2 h at 6000 × g. The supernatant was used as a cell-free system for incubation experiments.

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9) The stereochemistry of folding of the precursor may also be inferred from the sense of chirality at C(6) [25].

4. *Incorporation of [16,16,16,16′,16′,16′-$^2H_6$]Lycopene* (**6**). Each incubation run contained a soln. of **6** (20 μg) in EtOH (0.1 ml), mevinolin (**16**) (10 μg), and the cell-free system (3 ml; containing *ca.* 500 mg of protein), made up to 4 ml with 0.1M *Tris*·HCl buffer (pH 7.0). Four hundred such incubation runs were continually illuminated and shaken over a 20 h period at 30°.

5. *Isolation and Characterization of Labeled β,β-Carotene* (**17**). After termination of the incubation by addition of a quantity of MeOH and thorough mixing with a *Vortex* mixer, the contents were filtered over *Celite*, and the filter cake was extracted with acetone until no more color was apparent. The isolated carotenoids were saponified, separated into epi- and hypophases, and the carotene hydrocarbons were separated by CC on Al_2O_3 (*Woelm*, neutral; hexane/Et_2O 19:1). Further purification from the (*Z*)-isomer by HPLC (*Spherisorb S5*, NH_2; hexane/EtN(i-Pr)$_2$ 99.9:0.1; 0.5 ml/min; detection at 445 nm (*Fig. 3*) provided crystalline **17** (5 mg). CD (*Fig. 5*; EPA; 5:5:2): qualitative. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.029 (*s*, Me(16,16′,17,17′)); *ca.* 1.47 (*m*, H_2–C(2,2′)); *ca.* 1.63 (*m*, H_2–C(3,3′)); 1.720 (*s*, Me(18,18′)); 1.97 (*s*, Me(19,19′,20,20′)); 2.02 (*m*, H_2–C(4,4′)); 6.11–6.19 (*m*, Me(7,7′,8,8′,10,10′)); 6.265 (*m*, H–C(14,14′)); 6.355 (*d*, *J* = 15, H–C(12,12′)); 6.618–6.683 (*m*, H–C(11,11′,15,15′)). ^{2}H-NMR (*Fig. 4*; 61.4 MHz, $CDCl_3$): 1.035 (*s*, CD_3(1,1′)).

6. *(±)-[3′,3′,3′-$^2H_3$]MVA* (**14**). 6.1. *Dimethyl 2,2′-(1,3-Oxathiolane-2,2-diyl)bisacetate Dimethyl Ester* (**8**). To a soln. of dimethyl 3-oxopentanedioate (43.87 g) in CH_2Cl_2 (250 ml) was added, with stirring, first 2-mercaptoethanol (29.68 g) and then $BF_3 \cdot Et_2O$ (42.38 g). Stirring was continued for 8 h at r.t. The mixture was then diluted with Et_2O (400 ml) and H_2O (200 ml). After 15 min, the aq. phase was separated, and the Et_2O layer washed with aq. $NaHCO_3$ and then with aq. NaCl until neutral. The oil obtained, after drying (Na_2SO_4) and solvent removal, crystallized after a short time: **8** (52.5 g, 89%). M.p. 45°. IR ($CHCl_3$): 2958*w*, 1740*s*, 1438*s*, 1342*m*, 1195*w*, 1175*m*, 1080. ^{1}H-NMR (80 MHz, $CDCl_3$): 3.06 (*t*, *J* = 6.2, 6 H, 2 H–C(4)); 3.2 (*AB*, *J* = 18, 4 H); 3.69 (*s*, 2 MeO); 4.19 (*t*, *J* = 6, 2 H–C(5)). MS: 234 (6, M^+), 203 (3), 175 (11), 161 (33), 143 (16), 101 (25), 60 (100), 59 (35), 45 (14). Anal. calc. for $C_9H_{14}O_5S$ (234.27): C 46.14, H 6.02, S 13.69; found: C 46.21, H 5.90, S 13.69.

6.2. *2,2′-(1,3-Oxathiolane-2,2-diyl)bisethanol* (**9**). $LiAlH_4$ (10 g) was added to THF (120 ml) under an inert atmosphere. A soln. of **8** (30.86 g) in THF (250 ml) was added dropwise with stirring. Stirring was continued for 12 h, and then hydrolysis was performed with MeOH/H_2O 1:1 (30 ml), followed by 15% aq. NaOH (15 ml). After addition of H_2O (45 ml), stirring was continued for a further 30 min, and the mixture was then filtered over *Celite*; washing with THF. After evaporation of the org. phase, the residue obtained was dried *in vacuo.* Compound **9** was obtained as an oil in quant. yield. ^{1}H-NMR (80 MHz, $CDCl_3$): 2.17 (*t*, *J* = 6, 2 H–C(2′,2″)); 3.07 (*s*, 2 OH); 3.08 (*t*, *J* = 6, 2 H–C(4)); 3.81 (*t*, *J* = 6, 2 H–C(1′,1″)); 4.19 (*t*, *J* = 6, 2 H–C(4)). MS: 196 (6, $[M + 18(NH_3)]^+$),179 (24, $[M + 1]^+$), 169 (18), 136 (25), 120 (5), 102 (27), 85 (6), 35 (12). Anal. calc. for $C_7H_{12}OS$ (178.25): C 47.17, H 6.79, S 17.99; found: C 47.28, H 7.01, S 17.76.

6.3. *2,2-Bis[2-(tetrahydropyran-2-yloxy)ethyl]-1,3-oxathiolane* (**10**). 3,4-Dihydro-2*H*-pyran (33.73 g) and TsOH (0.5 g) were added at 0°, with powerful stirring, to a soln. of **9** (23.52 g) in CH_2Cl_2 (200 ml). After the reaction was complete, the soln. was diluted with Et_2O (300 ml), and then washed with aq. $NaHCO_3$ and aq. NaCl. After drying, solvent removal, and filtration through deactivated silica gel (hexane/Et_2O 3:2), **10** was obtained as a colorless oil (42 g, 92%). ^{1}H-NMR (80 MHz, $CDCl_3$): 1.5–1.8 (*m*, 6 CH_2 (tetrahydropyran)); 2.2 (*t*, 2 H–C(2′,2″)); 3.05 (*t*, 2 H–C(4)); 3.4–4.0 (*m*, 2 H–C(1′,1″), 2 CH_2 (tetrahydropyran)); 4.15 (*t*, 2 H–C(5)); 4.55 (*m*, 2 CH (tetrahydropyran)).

6.4. *1,5-Bis(tetrahydropyran-2-yloxy)pentan-3-one* (**11**). A soln. of **10** (14 g) in MeCN/H_2O 4:1 (150 ml) was treated with $CaCO_3$ powder (8 g) and then with $HgCl_2$ (24 g), stirred for 2 h at r.t., and filtered over *Celite*. The filtrate was treated with aq. NH_4OAc and extracted with Et_2O. After the usual workup, **11** (10.41 g, 90%) was obtained. IR (film): 2940*s*, 1715*s*, 1350*m*, 1200*m*, 1120*m*, 1035*m*. ^{1}H-NMR (80 MHz, $CDCl_3$): 1.48–1.80 (*m*, 6 CH_2 (tetrahydropyran)); 2.75 (*t*, 2 H–C(2,4)); 3.5–4.15 (*m*, 2 H–C(1,5), 2 CH_2 (tetrahydropyran)); 4.55 (*m*, 2 CH (tetrahydropyran)).

6.5. *1,5-Bis(tetrahydropyran-2-yloxy)-3-[$^2H_3$]methylpentan-3-ol* (**12**). To a *Grignard* soln. prepared from Mg (1 g) and CD_3I (5 g) in ether (70 ml) was added rapidly at 0° a soln. of **11** (7.5 g) in Et_2O (50 ml), and the mixture was refluxed for 2.5 h. After hydrolysis with aq. NH_4Cl and the usual workup, the product was chromatographed (silica gel; hexane/Et_2O 9:1) to give **12** (7.2 g, 90%). Colorless oil. ^{1}H-NMR (80 MHz, $CDCl_3$): 1.49–1.76 (*m*, 6 CH_2 (tetrahydropyran)); 1.85 (*t*, *J* = 6, 2 H–C(2,4)); 3.52–3.93 (*m*, 9 H); 4.59 (*m*, 2 CH (tetrahydropyran)).

6.6. *3-[$^2H_3$]Methylpentane-1,3,5-triol* (**13**). A soln. of **12** in MeOH (20 ml) was treated with TsOH (0.23 g) and heated under reflux for 4 h. After cooling, $NaHCO_3$ (0.21 g) was added, and the mixture was diluted with $CHCl_3$ (30 ml). After stirring for 20 min, the soln. was filtered through a layer of $MgSO_4$ and solvent was removed. CC on silica gel ($CHCl_3$) provided **13** (0.55 g, 85%). Viscous oil. ^{1}H-NMR (90 MHz, C_5D_5N): 2.10 (*m*, 2 H–C(2,4)); 4.15 (*t*, 2 H–C(1,5)); 5.75 (*s*, 3 OH).

6.7. *(±)-4-Hydroxy-4-[$^2H_3$]methyltetrahydropyran-2-one* (**14**, [$^2H_3$]MVL). A soln. of **13** (0.77 g) in MeOH (7 ml) was added to Ag_2CO_3/*Celite* (*Fétizon et al.* [29]) in benzene (300 ml). MeOH was distilled off, and H_2O was removed azeotropically with benzene. After refluxing for 6 h, the mixture was filtered, solvent was removed, and the product was distilled in a *Kugelrohr* at 110°/0.2 Torr to yield **14** (0.5 g). Clear liquid. IR ($CHCl_3$): 3603*w*, 3016*s*, 2227*w*, 1728*s*, 1404*w*, 1265*m*, 1234*m*, 1076*m*. ^{1}H-NMR (80 MHz, $CDCl_3$): 1.83–1.98 (*m*, 2 H–C(4)); 2.56–2.62 (*m*, 2 H–C(2)); 4.25–4.69 (*m*, 2 H–C(5)).

7. *[$^2H_3$]DMAA* (**21**) *and [$^2H_3$]DMAPP* (**23**). 7.1. *[4,4,4-$^2H_3$]But-2-yn-1-ol* (**19**). $LiNH_2$ (12 g) was placed in a flame-dried four-necked flask under Ar, and dried NH_3 (50 ml) was condensed onto it at −70°. Compound **18** was added dropwise over 15 min. After 45 min, CD_3I (25 g) was slowly added with continual stirring. After 3 h, the NH_3 was allowed to evaporate, and the residue was taken up in H_2O (70 ml), sat. with NaCl, and continuously extracted with Et_2O in a *Soxhlet* apparatus. After drying and distillation through a 10-cm *Vigreux* column, **19** (6.3 g, 50%) was obtained. B.p. 82°/100 Torr. ^{1}H-NMR (90 MHz, CCl_4): 3.6 (*s*, OH); 4.1 (*s*, 2 H–C(2)).

7.2. *3-Iodo[4,4,4-$^2H_3$]but-2-en-1-ol* (**20**). A suspension of $LiAlH_4$ (4.85 g) in THF (1 l) was treated with $NaOCH_3$ (97%, 14.2 g) and heated under reflux for 30 min. It was then allowed to cool to r.t., and a soln. of **19** (4.66 g) in THF (20 ml) was added dropwise. Heating under reflux was resumed for 3 h. After cooling to −70°, it was treated slowly with I_2 (62 g) in THF (140 ml). After stirring for 90 min at the same temp., the cooling bath was removed, and the mixture was hydrolyzed with H_2O and half-sat. NaCl soln. After extraction with H_2O and washing with thiosulfate soln. and sat. NaCl soln., it was dried ($MgSO_4$), filtered, and evaporated. CC on silica gel (CH_2Cl_2/Et_2O 9:1) produced **20** (11.18 g, 87%).

7.3. *(*E*)-3-Methyl[4,4,4-$^2H_3$]but-2-en-1-ol* (**21**). A suspension of CuI (28 g) in Et_2O (300 ml) was slowly treated with MeLi soln. in Et_2O (1.6M, 185 ml) at −15° over a 1 h period. A clear soln. resulted. To this was added dropwise under Ar a soln. of **20** (11.9 g) in Et_2O (30 ml), and the mixture was allowed to stir for 4.5 d at 0°. Workup was by careful addition of MeOH (4.5 ml) and H_2O (10.5 ml). The precipitate was separated by filtration over *Celite*. After extraction, washing, and solvent removal, the product was chromatographed (silica gel; CH_2Cl_2/Et_2O 4:1). One distillation of the product in a *Kugelrohr* at 100°/100 Torr gave **21** (3.72 g, 71%).

7.4. *(*E*)-1-Bromo-3-methyl[4,4,4-$^2H_3$]but-2-ene* (**22**). A soln. of **21** (3 g) in Et_2O (120 ml) was treated at 0° with PBr_3 (1.7 ml) and stirred for 1 h at r.t. After addition of dil. aq. $NaHCO_3$, it was washed until neutral, dried ($MgSO_4$), filtered, and concentrated. The subsequent *Kugelrohr* distillation provided **22** (3.4 g, 66%). ^{1}H-NMR (200 MHz, $CDCl_3$): 1.734 (*s*, Me–C(3)); 4.019 (*d*, *J* = 8.5, 2 H–C(1)); 5.530 (*t*, *J* = 7, H–C(2)).

7.5. *[$^2H_3$]DMAPP* (**23**). Prepared by the method of [18]. ^{1}H-NMR (200 MHz, D_2O/NH_4OD): 1.73 (*s*, Me–C(3)); 4.46 (br. *s*, 2 H–C(1)); 5.46 (br. *s*, H–C(2)).

8. *[$^2H_3$]GA* (**25**) *and [$^2H_3$]GPP* (**27**). 8.1. *Methyl (2*E*,6*E*)-3,7-Dimethyl[8,8,8-$^2H_3$]octa-2,6-dienoate* (**24**). To a suspension of NaH (55% in mineral oil, 459 mg) in THF (25 ml) at 0° was added, dropwise, methyl acetoacetate (1.08 ml). After 10 min, the clear soln. obtained was treated with BuLi soln. in hexane (1.6M, 6.4 ml). After 10 min, bromide **22** (1.6 g) was added dropwise. After 10 min stirring at r.t., it was once more cooled to 0° and treated with diethyl chlorophosphate (1.52 ml). After 2 h stirring at r.t., the mixture was added dropwise to a soln. of Me_2CuLi at −70°. (This had been prepared previously by dropwise addition of MeLi soln. (25 ml) to a suspension of CuI (3.8 g) in Et_2O (25 ml).) After allowing the mixture to warm to 0°, it was treated with aq. NH_4Cl and extracted with Et_2O. After washing with a small quantity of NH_3/aq. NaCl, drying, filtering, and CC (silica gel; hexane/Et_2O 7:3), the oil obtained was distilled in a *Kugelrohr* at 130°/10 Torr to yield **24** (1.35 g, 73%). Colorless oil. IR ($CHCl_3$): 2956*m*, 2222*w*, 2186*w*, 1715*s*, 1650*s*, 1438*s*, 1150*s*. ^{1}H-NMR (200 MHz, $CDCl_3$): 1.60 (*s*, Me–C(7)); 2.16 (br. *s*, Me–C(3), 2H–C(4,5)); 3.69 (*s*, MeO); 5.07 (br. *s*, H–C(2)); 5.67 (*s*, H–C(6)).

8.2. *(2*E*,6*E*)-3,7-Dimethyl[8,8,8-$^2H_3$]octa-2,6-dien-1-ol* (**25**). An ice-cooled soln. of **24** (1.134 g) in THF (120 ml) was treated with a soln. of diisobutylaluminium hydride (DIBAH, 1M, 17 ml) in hexane, and stirred for 10 min. After hydrolysis with an equiv. volume of H_2O and further stirring for 1 h, the mixture was diluted with Et_2O, saturated with brine, and the phases were separated. The org. layer was washed thoroughly with dil., aq. NaCl; drying ($MgSO_4$), solvent removal, and distillation in a *Kugelrohr* provided **25** (1.145 g). Colorless oil. B.p. 140°/10 Torr. IR ($CHCl_3$): 3610*m*, 2922*s*, 2222*w*, 2190*w*, 1668*w*, 1415*w*, 1380*m*, 980*s*. ^{1}H-NMR (200 MHz, $CDCl_3$): 1.60, 1.68 (2*s*, Me–C(3,7)); 2.08 (*s*, 2H–C(4,5), OH); 5.09 (br. *s*, H–C(2)); 5.42 (*t*, *J* = 7, H–C(6)). MS: 126 (5, $[M - CH_2OH]^+$), 93 (13), 72 (75), 40 (100).

8.3. *(2*E*,6*E*)-1-Chloro-3,7-dimethyl[8,8,8-$^2H_3$]octa-2,6-diene* (**26**). A soln. of **25** (1.1 g) and thoroughly dried Ph_3P (2.42 mg) in CCl_4 (7 ml) was heated under reflux for 2 h. After cooling, it was diluted with Et_2O (15 ml) and cooled with stirring to 0°, followed by filtration, solvent removal, and *Kugelrohr* distillation to yield **26** (820 mg, 78%). B.p. 60°/0.2 Torr. ^{1}H-NMR (90 MHz, CCl_4): 1.60, 1.70 (2*s*, Me–C(3,7)); 2.10 (*s*, 2 H–C(4,5));

4.00 (*d*, *J* = 7, 2 H–C(1)); 5.05 (br. *s*, H–C(2)); 5.40 (*t*, H–C(6)). ^{2}H-NMR (61.4 MHz, $CHCl_3$): 1.67 (*s*, CD_3(8)).

8.4. *(2E,6E)-3,7-Dimethyl[8,8,8-$^2H_3$]octa-2,6-dienyl Diphosphate* ([$^2H_3$]GPP; **27**). Preparation analogous to that of **23**. ^{1}H-NMR (200 MHz, D_2O/NH_4OD): 1.64 (*s*, Me–C(7)); 1.73 (*s*, Me–C(3)); 2.14 (*m*, 2 H–C(4,5)); 4.48 (*dd*, *J* = 6.6, 2 H–C(1)); 5.24 (*m*, H–C(6)); 5.48 (br. *t*, *J* = 7, H–C(2)). ^{31}P-NMR (81 MHz, D_2O/ND_4OD): –9.51 (*d*, *J* = 21, P(1)); 5.70 (*d*, *J* = 22, P(2)).

9. *[$^2H_3$]FA* (**29**) *and [$^2H_3$]FPP* (**31**). 9.1. *Methyl (2E,6E,10E)-3,7,11-Trimethyl[12,12,12-$^2H_3$]dodeca-2,6,10-trienoate* (**28**). A suspension of NaH (55% in mineral oil, 103 mg) in THF (5.5 ml) was added with ice-cooling to methyl acetoacetate (250 mg) and then stirred for 10 min, after which BuLi in hexane (1.6M, 1.35 ml) was added. After a further 10 min, **26** (280 mg) was added, and the mixture was stirred for 1 h at 0° and then for 1 h at r.t. After hydrolysis with a little H_2O and the usual workup, CC was performed (silica gel; hexane/Et_2O 4 : 1). It was possible to recover unreacted **26** (84 mg, 30%). The enolized *β*-keto ester (245 mg, 64%) was isolated by *Kugelrohr* distillation at 100°/0.01 Torr. The product was then added dropwise to a suspension of NaH (48 mg) in THF (3.5 ml). After 20 min at 0°, it was treated with $ClPO(OEt)_2$ (194 mg). After stirring for 2 h at r.t., it was then cooled to –78° and treated with the soln. prepared from CuI (430 mg) and MeLi (2.82 ml) (as described for **24**). After an analogous workup, we obtained **28** (157 mg, 61%). Colorless oil. B.p. 100°/0.1 Torr. ^{1}H-NMR (90 MHz, $CDCl_3$): 1.60 (*s*, Me–C(7,11)); 2.0–2.18 (*m*, Me–C(3), 2 H–C(4,8,9)); 3.68 (*s*, MeO); 5.10 (*m*, H–C(6,10)); 5.58 (br. *s*, H–C(2)).

9.2. *(2E,6E,10E)-3,7,11-Trimethyl[12,12,12-$^2H_3$]dodeca-2,6,10-trien-1-ol* (**29**). DIBAH reduction of **28** (157 mg) as described for **25** produced **29** (147 mg).

9.3. *[$^2H_3$]Farnesyl Chloride* (**30**). A soln. of *N*-chlorosuccinimide (98 mg) in CH_2Cl_2 (3 ml) was treated at –30° with Me_2S (0.54 ml). After cooling to –40°, a soln. of **29** (140 mg) in CH_2Cl_2 (1 ml) was added to it dropwise. The mixture was allowed to warm to 0° over 1 h. After a further 1 h stirring at this temp., it was diluted with Et_2O and worked up in the usual manner. Isolated **30** was used without further purification in the following step.

9.4. *(2E,6E,10E)-3,7,11-Trimethyl[12,12,12-$^2H_3$]dodeca-2,6,10-trienyl Diphosphate* ([$^2H_3$]FPP; **31**). Prepared as described for **23**. ^{1}H-NMR (400 MHz, D_2O): 1.62 (*s*, Me–C(7,11)); 1.72 (*s*, Me–C(3)); 2.04–2.17 (*m*, 2 H–C(4,5,8,9)); 5.20 (*m*, H–C(6,10)); 5.50 (*m*, H–C(2)). ^{31}P-NMR (81 MHz, D_2O/ND_4OD): –9.28 (br. *s*, P(1)); –5.58 (br. *s*, P(2)).

9.5. *Synthesis of* **29** (*via Scheme 5*). *(E)-3-Methyl-1-(phenylsulfonyl)[4,4,4-$^2H_3$]but-2-ene* (**32**). A soln. of **21** (1.24 g) in Et_2O (60 ml) was converted to bromide **22** with PBr_3 (1.6 g) in Et_2O (30 ml) as described under *7.4*. It was then treated immediately with $NaSO_2Ph$ (2.3 g) in DMF (12 ml). After stirring overnight at r.t. and the usual workup, crystalline **32** (800 mg) was obtained. CC of the mother liquors (silica gel; CH_2Cl_2/Et_2O 9 : 1) gave a further 152 mg of **32** (total yield 32%). ^{1}H-NMR (200 MHz, $CDCl_3$): 1.31 (*s*, Me–C(3)); 3.79 (*d*, *J* = 8, 2 H–C(1)); 5.19 (*d*, *J* = 8, H–C(2)); 7.55–7.61 (*m*, 3 H); 7.86–7.89 (*m*, 2 H).

9.6. *Methyl 4-Methyl-2-(phenylsulfonyl)[5,5,5-$^2H_3$]pent-3-enoate* (**33**). A soln. of **32** (866 mg) in THF (40 ml) was treated dropwise at –20° with a soln. of BuLi in hexane (1.6M, 3.6 ml). After 10 min, ClCOOMe (1 ml, 1.23 g) was added. After stirring for 2 h at r.t., it was worked up in the usual manner. CC of the product (silica gel; Et_2O/hexane 7 : 3) gave **33** (760 mg). ^{1}H-NMR (90 MHz, $CDCl_3$): 1.46 (*s*, Me–C(3)); 3.70 (*s*, MeO); 4.75 (*d*, *J* = 9, H–C(1)); 5.28 (*d*, *J* = 9, H–C(2)).

10. *[$^2H_3$]GGA* (**39**). 10.1. *Methyl (2E,6E,10E,14E)-13-(Methoxycarbonyl)-3,7,11,15-tetramethyl-13-(phenylsulfonyl)[16,16,16-$^2H_3$]hexadeca-2,5,10,14-tetraenoate* (**37**). Prepared as described in [21]. To the *π*-allyl complex of methyl farnesate (814 mg) in Et_2O (90 ml) was added Ph_3P (394 mg). After 10 min stirring, Et_2O was evaporated with N_2, the residue dried *in vacuo*, taken up in THF (45 ml), and renewed addition of Ph_3P (394 mg) gave *Soln. I*. To a suspension of NaH (in mineral oil, 136 mg) in THF (40 ml) was added dropwise a soln. of **33** (814 mg) in THF (8 ml) at r.t. This was stirred until a clear soln. had been produced, giving *Soln. II*, which was added in one portion to *Soln. I*, and the mixture was stirred at r.t. for 5 h. The mixture was worked up by pouring into half-sat. aq. NaCl and extracting with Et_2O. The combined org. fractions were washed with H_2O, dried ($MgSO_4$), filtered, and the solvent was removed. CC (silica gel; hexane/Et_2O 3 : 2) yielded **37** (547 mg, 34%). Colorless oil. ^{1}H-NMR (90 MHz, $CDCl_3$): 1.25 (*t*, *J* = 7.5, MeO); 1.40 (*s*, Me–C(15)); 1.56 (*s*, Me–C(7,11)); 1.93 (br. *s*, 2 CH_2); 2.13 (*s*, Me–C(3), 2 CH_2); 2.95–3.05 (2 br. *s*, 2 H–C(12)); 3.66 (*s*, MeO); 4.13 (*q*, *J* = 7.5, CH_2O); 5.10 (*m*, H–C(6,10)); 5.30 (*s*, H–C(14)); 5.63 (*s*, H–C(2)), 7.36–7.63 (*m*, 2 H), 7.76–8.0 (*m*, 2 H).

10.2. *(2E,6E,10E,14E)-3,7,11,15-Tetramethyl-13-(phenylsulfonyl)[16,16,16-$^2H_3$]hexadeca-2,6,10,14-tetraenoate* (**38**). A soln. of **37** (547 mg), 4-aminophenol (270 mg), and Cs_2CO_3 (120 mg) in DMF (6 ml) was stirred at 85° for 1.5 h. After cooling, the mixture was poured into half-sat. aq. NaCl, extracted with Et_2O, and

the combined org. phases were washed with dil. HCl and aq. NaCl, and dried ($MgSO_4$). After filtration and solvent removal, the product was chromatographed (silica gel; hexane/AcOEt 3:1) to yield **38** (310 mg, 64%). IR ($CHCl_3$): 2935*m* (br.), 2984*m*, 1708*s*, 1648*m*, 1450*m*, 1385*w*, 1305*s*, 1145*s*, 1085*m*. ^{1}H-NMR (200 MHz, $CDCl_3$): 1.14 (*s*, Me–C(15)); 1.28 (*t*, *J* = 7.5, MeO); 1.51 (*s*, 3 H); 1.57 (*s*, 3 H); 1.88–2.12 (*m*, 2 H–C(4,5,8,9)); 2.15 (*s*, Me–C(3)); 2.85 (*d*, *J* = 15, 2 H–C(12)); 3.84 (*m*, H–C(13)); 4.14 (*q*, *J* = 7.5, CH_2O); 4.89 (*d*, *J* = 10, H–C(14)); 5.60 (*m*, H–C(6,10)); 5.66 (*s*, H–C(2)); 7.4–7.7 (*m*, 3 H); 7.8–7.9 (*m*, 2 H).

10.3. *(2*E*,6*E*,10*E*,14*E*)-3,7,11,15-Tetramethyl-13-(phenylsulfonyl)[16,16,16-$^2H_3$]hexadeca-2,6,10,14-tetraen-1-ol* (**39**). A soln. of **38** (600 mg) in THF (40 ml) was treated dropwise at –20° with a soln. of DIBAH in hexane (1M, 3 ml). After stirring for 1 h at this temp., it was hydrolyzed with H_2O and extracted with Et_2O and worked up as usual. Chromatography (silica gel; Et_2O/hexane 7:3) yielded **39** (495 mg, 91%). Colorless oil. ^{1}H-NMR (200 MHz, $CDCl_3$): 1.13 (*s*, Me–C(15)); 1.43 (*s*, OH); 1.51 (*s*, Me–C(7)); 1.57 (*s*, Me–C(13)); 1.68 (*s*, Me–C(11)); 1.89–2.139 (*m*, 2 H–C(4,5,8,9)); 3.86 (*m*, H–C(13)); 4.16 (*d*, *J* = 7, 2 H–C(1)); 4.89 (*d*, *J* = 11, H–C(14)); 5.00–5.20 (*m*, 2 H–C(12)); 5.41 (*t*, *J* = 7, H–C(2)); 7.47–7.62 (*m*, 3 H); 7.81–7.86 (*m*, 1 H).

10.4. *(2*E*,6*E*,10*E*,14*E*)-3,7,11,15-Tetramethyl[16,16,16-$^2H_3$]hexa-2,6,10,14-tetraen-1-ol* ([$^2H_3$]GGA; **40**). The desulfonation of **39** was carried out with Li/$EtNH_2$ as described in [26]. ^{1}H-NMR (200 MHz, $CDCl_3$): 1.60 (*s*, Me–C(7,11,15)); 1.68 (*s*, Me–C(3)); 4.15 (*d*, *J* = 6.8, 2 H–C(1)); 5.11 (br. *s*, H–C(6,10,14)); 5.42 (*m*, H–C(2)). ^{2}H-NMR (61.4 MHz, $CHCl_3$): 1.65 (*s*, CD_3(16)). MS: 293 (9, M^+), 277 (27, $[M-H_2O]^+$), 276 (100), 220 (23), 208 (84), 194 (30), 189 (19), 180 (29), 166 (35), 152 (59), 149 (58), 140 (69), 135 (42), 126 (41), 123 (53), 121 (62), 109 (40), 95 (43), 81 (70), 72 (45).

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