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ω-Di-(trideuteromethyl)-tocotrienols as probes for membrane orientation and dynamics of tocotrienols

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The study of the dynamic and structural role of lipids in membranes is commonly accomplished using ^{2}H NMR and neutron diffraction. This necessarily requires the availability of specifically deuterated lipids. Our interest in the behaviour of tocopherols and tocotrienols in phospholipid membranes has shown that the tocotrienols, unsaturated forms of vitamin E, have very different effects on membrane properties as judged by differential scanning calorimetry (DSC). We report here the preparation of deuterated forms of α-, β-, and γ-tocotrienols where the terminal methyl groups on the isoprenoid side chains have been replaced with trideuteromethyl groups, thus incorporating six deuterium atoms per molecule. Starting from the naturally occurring tocotrienols, the terminal alkene can be selectively hydrobrominated with NBS, transformed to a diol and oxidatively cleaved to give a chain truncated aldehyde. The full chain length is then restored by reaction with the ylide formed by deprotonation of triphenyl-(1,2,2,2-tetradeutero-1-trideuteromethyl-ethyl)-phosphonium bromide.

Keywords: α-tocotrienol; β-tocotrienol; γ-tocotrienol; deuteration; trideuteromethyl; membrane probes; ^{2}H-NMR; neutron diffraction

Introduction

The tocotrienols have attracted recent interest from cell biologists and nutritionists due to their promising biological activities, particularly with regards to apoptosis and cessation of cancer cell growth,[1–8] and protection of neurological damage in post-eschemic models of stroke.[9–11] The biological activities of tocotrienols do not appear to be obviously connected to antioxidant activity even though tocotrienols are accepted to be equal or better antioxidants – by various measures – than tocopherols.[12–16]

A recent investigation of the effects of tocopherols and tocotrienols on membrane phospholipids structures by differential scanning calorimetry (DSC)[17] showed that the tocotrienols have significantly greater effects on phospholipids phase transitions than do tocopherols. These effects were concluded to be due to the tocotrienol's contribution of negative curvature to phospholipid bilayers. In this light, we have begun a collaborative project to explore the behaviour of tocopherols and tocotrienols in phospholipids of differing degrees of fluidity (unsaturation) and composition. The chief techniques for assessing such behaviour are ^{2}H-NMR[18] and neutron diffraction.[19] Both of these techniques demand that molecules of interest include specifically situated deuterium atoms that act as reporters of the relative depths with respect to the bilayer midplane as well as their dynamics as reflected in NMR-order parameters.

It is anticipated that the side chain of tocotrienols is more conformationally mobile than that of tocopherols due to a reduced energy barrier for bond rotation around the allylic methylenes next to the double bonds. Such hypermobility is reflected in the larger effect that tocotrienols have versus tocopherols on phospholipid phase behaviour as seen by DSC.17 We have previously prepared a C5–CD_3 labeled tocotrienol that reports on the position and behaviour of the chroman head in lipid membranes[20] but it is also necessary to observe the other end of the molecule, i.e. the terminus of the isoprenoid side chain. Substitution of the chain terminal methyl groups with CD_3 groups would satisfy these needs. We report here the preparation of chain terminal trideuteromethyl-containing α-, β-, and γ-tocotrienols for use as ^{2}H-NMR and neutron diffraction probes of the membrane behaviour of tocotrienols.

Results

As tocotrienols can be easily retrieved from tocol-containing concentrates of palm oil,[20] we preferred to start from these materials rather than build up the structures in a more complex synthesis.[21–24] This choice of starting materials necessitates the selective functionalization of the terminal double bond on the side chain, excising the propylidene group and introducing some functionality that would allow the addition of a deuterated replacement. We found precedence for the selective functionalization of a terminal isoprenoid alkene in the work of Chen *et al.*[25] who used wet *N*-bromosuccinimide (NBS) to form a

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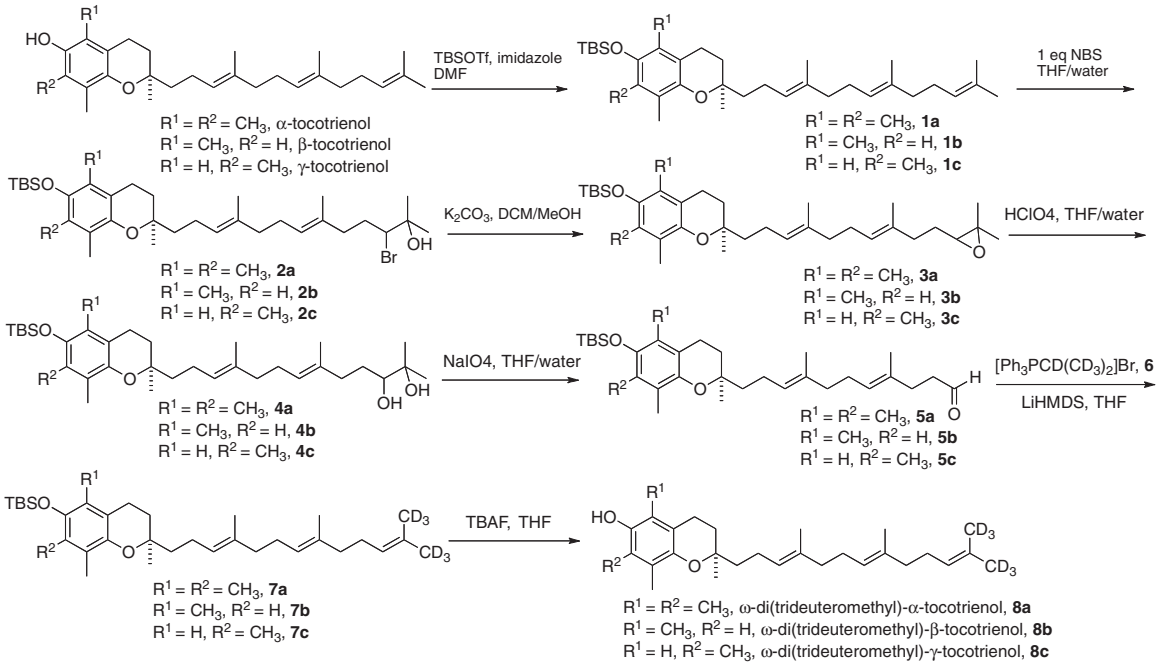

Figure 1. Synthetic route for the preperation of chain terminally deuterated tocotrienols starting from natural source tocotrienols.

bromohydrin from the alkene, which easily transformed to the epoxide under basic conditions and a diol upon ring opening.[26] Figure 1 illustrates our synthetic route.

Our work began by combining a slight excess of NBS with silyl-protected α-tocotrienol **1a** in a *tert*-butanol/water solution as suggested by Chen *et al.*[25] During this step, some starting α-tocotrienol always remained as determined by TLC. Yields for this conversion could not be accurately calculated until the diol **4a** was formed since the diol is more stable than both the bromohydrin and the epoxide, and thus could be isolated. The formation of the epoxide and hydrolysis to the diol appeared to go to completion as judged by TLC, but the overall yield of **4a** from **1a** was only 25–30%. It was deduced that formation of the bromohydrin was the yield-limiting step.

Several test reactions were made with varying amounts of excess NBS (1.1–3.5 equivalents) and it was found that 1.1 equivalents of NBS (in *tert*-butanol/water) provided the best overall yield. Any further increase in the amount of NBS generated more side products from reaction with the remaining double bonds to form a bromohydrin/epoxide/diol mixture, suggested by mass spectrometry, but not confirmed by a full characterization. NBS is thus selective for the least bulky, terminal alkene. The formation of the bromohydrin with 1.1 equivalents of NBS was repeated again using THF instead of *tert*-butanol, and the total yields improved further with fewer byproducts as judged by TLC. After epoxidation and hydrolysis to the diol **4a**, the yield for the three steps had improved slightly to 35%.

Diol **4a** was cleaved using sodium periodate.[25] The aldehyde **5a** was used crude in the next step since attempts to purify **5a** via silica gel column chromatography resulted in its decomposition. Thus, crude aldehyde **5a** was reacted with the ylide of the hexadeuterated species **6**[27,28] formed by treatment of **6** with lithium hexamethyldisilylamide (Li-HMDS) to afford **7a**. Finally, deprotection with TBAF provided hexadeuterated **8a** in high yield. By this method we could readily prepare several hundred milligrams of **8a** for use in spectroscopic studies. Analogous procedures were performed with β- and γ-tocotrienols to prepare terminally di(trideuteromethyl)-β-tocotrienol **8b**, and γ-tocotrienol **8c**.

Conclusion

Chain-terminally deuterated tocotrienols have been prepared in fair yields starting from the parent tocotrienols. Studies of the behaviour of these tocotrienols in phospholipids membranes by solid-state ^{2}H NMR and neutron diffraction are currently being conducted by Dr Stephen R. Wassall of the Department of Physics at Indiana University–Purdue University, Indianapolis and Dr Thad A. Harroun of the Department of Physics at Brock University, respectively. The results from these experiments will give further insight into the dynamics and structural role of tocotrienols in lipid membranes.

Experimental

Spectroscopic analysis of compounds was performed by ^{1}H, ^{2}H, and ^{13}C nuclear magnetic resonance (NMR) data obtained using a Bruker Avance DPX-300 Digital FT-NMR spectrometer at 300 MHz, 92 MHz, and 75 MHz, respectively. Cambridge Isotope Laboratories, Inc. deuterated chloroform (99.8% pure) was used as the solvent with the internal reference of residual chloroform (^{1}H = 7.24 ppm, ^{13}C = 77.0 ppm). Chemical shifts are reported in ppm (δ) (multiplicity, number of protons, assignment, and coupling constant in Hz). Multiplicity is designated using the following abbreviations: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet). Low resolution mass spectra (MS) were recorded on a Carlo Erba/Kratos GC/MS Concept 1S double focusing mass spectrometer interfaced to a Kratos DART acquisition system and a SUN SPARC workstation. Samples were

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introduced through a direct inlet system and ions were generated using electron impact (EI) at 70 eV, electrospray ionization (ESI), or fast atom bombardment (FAB) sources and are reported as *m/z* values for the parent peak and major fragments.

Terminally deuterated α-tocotrienol

*Synthesis of tert-butyl-dimethyl-[(R)-2,5,7,8-tetramethyl-2-((3E,7E)-4,8,12-trimethyl-trideca-3,7,11-trienyl)-chroman-6-yloxy]-silane **(1a)***

α-Tocotrienol (859.1 mg, 2.02 mmol) and imidazole (550.9 mg, 8.09 mmol) were dissolved in 3.5 mL anhydrous DMF under an argon atmosphere. *tert*-Butyldimethylsilyl trifluoromethanesulfonate (700 μL, 3.05 mmol) was added to the mixture and it was heated in an oil bath to 85°C with a calcium chloride drying tube for 15 h. The reaction mixture was then diluted with water and extracted using diethyl ether. The organic layer was dried with anhydrous sodium sulfate and concentrated under reduced pressure to afford a light yellow oil. Compound **1** was purified via column chromatography (DCM/hexane 3:1). (983.4 mg, 1.82 mmol, 90%), light yellow oil, $R_f = 0.72$ (DCM/hexane 3:1), ^{1}H NMR ($CDCl_3$) δ 5.20 (overlapping triplets, 3H, C = C<u>H</u>), 2.67 (t, 2H, C-4 <u>H</u>), 2.27 (m, 1H, C-3 <u>H</u>), 2.20–2.15 (m, 5H, C-1′ <u>H</u>), 2.20 (s, 3H, Ar-C<u>H</u>$_3$), 2.19 (s, 3H, Ar-C<u>H</u>$_3$), 2.15 (s, 3H, Ar-C<u>H</u>$_3$), 2.08 (m, 4H), 1.89 (m, 2H), 1.80 (s, 3H, C<u>H</u>$_3$), 1.71 (s, 9H, 3 C<u>H</u>$_3$), 1.70 (m, 1H, C-3 <u>H</u>), 1.37 (s, 3H, C-2 C<u>H</u>$_3$), 1.36 (m, 1H, C-1′ <u>H</u>), 1.15 (s, 9H, 3 C<u>H</u>$_3$), 0.22 (s, 6H, 2 C<u>H</u>$_3$); ^{13}C NMR ($CDCl_3$) δ 145.9, 144.1, 134.9, 131.0, 125.8, 124.5, 124.4, 124.2, 123.4, 122.7, 117.3, 74.2, 39.7, 39.5, 31.6, 26.8, 26.6, 26.1, 25.7, 23.8, 22.2, 20.9, 18.6, 17.6, 16.0, 15.8, 14.3, 13.4, 11.9, −3.4; MS (EI) *m/z* 538 (M^+, 100%), 279 (26.4%), 223 (11.1%), 73 (53.9%).

*Synthesis of (6E,10E)-3-bromo-13-[(R)-6-(tert-butyl-dimethyl-silanyloxy)-2,5,7,8-tetramethyl-chroman-2-yl]-2,6,10-trimethyl-trideca-6,10-dien-2-ol **(2a)***

To a solution of **1a** (370 mg, 0.69 mmol) in THF/water 7:1 (8 mL) at 0°C, NBS (122.2 mg, 0.69 mmol) was slowly added as a solid. The reaction mixture was stirred for 1 h and was then diluted with water for extraction with diethyl ether. After drying with anhydrous magnesium sulfate, the organic solution containing **2a** was concentrated under reduced pressure and remained crude for the next reaction. Yellow cloudy oil, $R_f = 0.21$ (DCM).

*Synthesis of tert-butyl-{;(R)-2-[(3E,7E)-10-(3,3-dimethyl-oxiranyl)-4,8-dimethyl-deca-3,7-dienyl]-2,5,7,8-tetramethyl-chroman-6-yloxy}-dimethyl-silane **(3a)***

Crude bromohydrin **2a** was dissolved in a saturated solution of potassium carbonate in methanol (7 mL). An additional 1 mL DCM was added to assist **2a** to go into solution and the mixture was allowed to stir at room temperature for 15 h. Later, it was extracted using DCM, dried with anhydrous magnesium sulfate, and concentrated to afford a crude epoxide **3a**. Yellow cloudy oil, $R_f = 0.52$ (DCM).

*Synthesis of (6E,10E)-13-[(R)-6-(tert-butyl-dimethyl-silanyloxy)-2,5,7,8-tetramethyl-chroman-2-yl]-2,6,10-trimethyl-trideca-6,10-diene-2,3-diol **(4a)***

400 mg of crude **3a** was dissolved in THF (1 mL) along with 3% $HClO_4$ (1 mL) and distilled water (750 μL). The reaction mixture was left stirring at room temperature for 4.5 h. It was then extracted with DCM, dried with anhydrous magnesium sulfate, and concentrated to yield crude **4a**. The diol was purified via column chromatography (Hexane/ethyl acetate 2:1). (138.2 mg, 0.24 mmol, 35% for three steps), clear yellow oil, $R_f = 0.36$ (Hexane/ethyl acetate 2:1), ^{1}H NMR ($CDCl_3$) δ 5.14 (overlapping triplets, 2H, C = C<u>H</u>), 3.34 (m, 1H, C<u>H</u>OH), 2.57 (t, 2H, C-4 <u>H</u>), 2.27 (m, 1H, C-3 <u>H</u>), 2.10–2.05 (m, 4H), 2.10 (s, 3H, Ar-C<u>H</u>$_3$), 2.08 (s, 3H, Ar-C<u>H</u>$_3$), 2.05 (s, 3H, Ar-C<u>H</u>$_3$), 2.00 (m, 2H), 1.80 (m, 4H), 1.61 (s, 3H, C<u>H</u>$_3$), 1.59 (s, 3H, C<u>H</u>$_3$), 1.47–1.41 (m, 3H, C-3 <u>H</u>), 1.25 (s, 3H, C-2 C<u>H</u>$_3$), 1.20 (s, 3H, C<u>H</u>$_3$), 1.15 (s, 3H, C<u>H</u>$_3$), 1.05 (s, 9H, 3 C<u>H</u>$_3$), 0.12 (s, 6H, 2 C<u>H</u>$_3$); ^{13}C NMR ($CDCl_3$) 145.8, 144.1, 134.8, 125.8, 125.0, 124.6, 124.5, 123.5, 122.6, 117.4, 78.3, 74.2, 73.0, 39.6, 39.4, 36.7, 31.6, 29.6, 26.5, 26.4, 26.1, 23.8, 22.2, 20.8, 18.6, 15.9, 15.8, 14.3, 14.2, 13.4, 11.9, −3.4; MS (FAB) *m/z* 572 (M^+, 15.1%), 554 (31.5%), 512 (20.6%), 279 (35.1%), 221 (16.8%), 149 (20.9%), 73 (76.6%), 57 (67.5%), 43 (100%).

*Synthesis of (4E,8E)-11-[(R)-6-(tert-butyl-dimethyl-silanyloxy)-2,5,7,8-tetramethyl-chroman-2-yl]-4,8-dimethyl-undeca-4,8-dienal **(5a)***

4a (520 mg, 0.91 mmol) was dissolved in THF/water 2:1 (12 mL), then sodium periodate (388.3 mg, 1.82 mmol) was added. The reaction was monitored by TLC and went on for 15 h. It was extracted using DCM and dried with anhydrous magnesium sulfate. Aldehyde **5a** was not chromatographed and remained crude for the next step. Clear yellow oil, $R_f = 0.79$ (Hexane/ethyl acetate 2:1), ^{1}H NMR ($CDCl_3$) δ 9.73 (bs, 1H, C<u>H</u>O), 5.13 (overlapping triplets, 2H, C = C<u>H</u>), 2.57 (t, 2H, C-4 <u>H</u>), 2.49 (t, 2H), 2.30 (t, 2H), 2.11 (m, 1H, C-3 <u>H</u>), 2.10 (overlapping singlets, 6H, 2 Ar-C<u>H</u>$_3$), 2.06 (s, 3H, Ar-C<u>H</u>$_3$), 2.00 (m, 4H), 1.80 (m, 4H), 1.60 (s, 3H, C<u>H</u>$_3$), 1.58 (s, 3H, C<u>H</u>$_3$), 1.35 (s, 1H, C-3 <u>H</u>),1.25 (s, 3H, C-2 C<u>H</u>$_3$), 1.05 (s, 9H, 3 C<u>H</u>$_3$), 0.12 (s, 6H, 2 C<u>H</u>$_3$); MS (EI) *m/z* 512 (M^+, 26.3%), 376 (100%), 279 (29.1%), 221 (15.3%), 73 (65.9%), 43 (72.3%).

*Synthesis of triphenyl-(1,2,2,2-tetradeutero-1-trideuteromethyl-ethyl)-phosphonium bromide **(6)**[27,28]*

2-Bromo-(1,1,1,2,3,3,3-$^2H_7$)-propane (4.1 g, 33.5 mmol) and triphenylphosphine (9.2 g, 35.2 mmol) were combined in a thick-walled vial and sealed with a screw cap. The mixture was heated to 150°C for 48 h. Product **6** was recrystallized from ethyl acetate/ethanol 20:1 and washed with pure ethyl acetate. (8.29 g, 21.1 mmol, 63%), white crystals, $R_f = 0.42$ (DCM/methanol 10:1), MS (ESI – positive) *m/z* 312 ($[M\text{-}Br]^+$, 100%).

*Synthesis of tert-butyl-dimethyl-[(R)-2,5,7,8-tetramethyl-2-((3E,7E)-13,13,13-trideutero-4,8-dimethyl-12-trideuteromethyl-trideca-3,7,11-trienyl)-chroman-6-yloxy]-silane **(7a)***

Phosphonium salt **6** (346.3 mg, 0.88 mmol) was dissolved in dry THF (10 mL) under an argon atmosphere and lithium bis(trimethylsilyl)amide (1.0 M in THF, 2.21 mL, 2.21 mmol) was added dropwise. The solution of ylide was stirred for 1 h, then a solution of crude **5a** (411.6 mg, 0.80 mmol) dissolved in dry THF (10 mL) was slowly added over the course of 40 min via cannula. After 15 h, the reaction mixture was quenched with ammonium chloride (10 mL) and **7a** was purified via column chromatography (DCM). (270.1 mg, 0.50 mmol, 54% for two steps), pale yellow oil, $R_f = 0.78$ (DCM), ^{1}H NMR ($CDCl_3$) δ 5.13 (overlapping triplets, 3H, C = C<u>H</u>), 2.56 (t, 2H, C-4 <u>H</u>), 2.16 (m, 1H, C-3 <u>H</u>),

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2.11–2.05 (m, 4H), 2.11 (overlapping singlets, 6H, 2 Ar-CH_3), 2.08 (s, 3H, Ar-CH_3), 2.00 (m, 4H), 1.83 (m, 2H), 1.72–1.48 (m, 2H), 1.60 (s, 6H, 2 CH_3), 1.26 (overlapping singlet and multiplet, 4H, C-2 CH_3, C-3 H), 0.88 (s, 9H, 3 CH_3), 0.14 (s, 6H, 2 CH_3); ^{2}H NMR (two drops of $CDCl_3$ in 600 μL $CHCl_3$) δ 1.64 (s, 3H, CD_3), 1.56 (s, 3H, CD_3); ^{13}C NMR ($CDCl_3$) δ 145.8, 144.1, 135.0, 134.9, 133.8, 133.6, 125.8, 124.4, 124.2, 123.5, 122.7, 117.4, 74.2, 39.7, 39.6, 39.5, 31.6, 26.7, 26.6, 26.1, 23.8, 22.2, 20.9, 18.6, 16.0, 15.8, 14.3, 13.4, 11.9, −3.4; MS (EI) *m/z* 544 (M^+, 100%), 279 (20.7%), 221 (10.1%), 73 (50.7%).

Synthesis of (R)-2,5,7,8-tetramethyl-2-((3E,7E)-13,13,13-trideutero-4,8-dimethyl-12-trideuteromethyl-trideca-3,7,11-trienyl)-chroman-6-ol ***(8a)***

7a (130 mg, 0.24 mmol) was dissolved in dry THF (5 mL) and tetrabutylammonium fluoride (1.0 M in THF, 1.19 mL, 1.19 mmol) was added dropwise. The solution was stirred at room temperature for 2 h and then quenched with 1 N HCl (2.5 mL). Product **8a** was purified via column chromatography (DCM/hexane 3:1). (70 mg, 0.16 mmol, 67%), pale yellow oil, R_f = 0.45 (DCM/hexane, 3:1), 97.6% isotopically pure, ^{1}H NMR ($CDCl_3$) δ 5.15 (overlapping triplets, 3H, C = CH), 4.24 (s, 1H, Ar-OH), 2.65 (t, 2H, C-4 H), 2.19–2.08 (m, 6H), 2.19 (s, 3H, Ar-CH_3), 2.16 (s, 3H, Ar-CH_3), 2.14 (s, 3H, Ar-CH_3), 2.02 (m, 4H), 1.82 (m, 2H), 1.70–1.50 (m, 2H), 1.64 (s, 3H, CH_3), 1.63 (s, 3H, CH_3), 1.27 (s, 3H, C-2 CH_3); ^{13}C NMR ($CDCl_3$) δ 145.5, 144.6, 135.0, 134.9, 131.0, 124.4, 124.2, 122.6, 121.1, 118.5, 117.3, 74.3, 39.7, 39.6, 39.5, 31.6, 26.7, 26.6, 23.7, 22.2, 20.7, 16.0, 15.9, 12.2, 11.8, 11.2; MS (EI) *m/z* 430 (M^+, 28.5%), 294 (43.1%), 205 (20.3%), 165 (100%), 75 (27.3%), 43 (22.4%).

Terminally deuterated β-tocotrienol

β-Tocotrienol was prepared from δ-tocotrienol isolated from palm oil concentrates as reported previously.[20]

Synthesis of tert-butyl-dimethyl-[(R)-2,5,8-trimethyl-2-((3E,7E)-4,8,12-trimethyl-trideca-3,7,11-trienyl)-chroman-6-yloxy]-silane ***(1b)***

β-Tocotrienol (1.06 g, 2.59 mmol) and imidazole (704.0 mg, 10.3 mmol) were dissolved in 3.5 mL anhydrous DMF under an argon atmosphere. *tert*-Butyldimethylsilyl trifluoromethanesulfonate (890 μL, 3.88 mmol) was added to the mixture and it was heated in an oil bath to 85°C with a calcium chloride drying tube for 15 h. The reaction mixture was then diluted with water and extracted using diethyl ether. The organic layer was dried with anhydrous sodium sulfate and concentrated under reduced pressure to afford a light yellow oil. Compound **1b** was purified via column chromatography (DCM/hexane 3:1). (670.0 mg, 1.28 mmol, 49%), light yellow oil, R_f = 0.65 (DCM/hexane 1:1), ^{1}H NMR ($CDCl_3$) δ 6.61 (s, 1H, Ar-H), 5.24 (overlapping triplets, 3H, C = CH), 2.71 (t, 2H, C-4 H), 2.28 (m, 1H, C-3 H), 2.26 (s, 3H, Ar-CH_3), 2.28-2.13 (m, 5H), 2.19 (s, 3H, Ar-CH_3), 2.12 (m, 4H), 1.92 (m, 2H), 1.81 (s, 3H, CH_3), 1.73 (s, 9H, 3 CH_3), 1.81–1.65 (m, 2H), 1.38 (s, 3H, C2-CH_3), 1.16 (s, 9H, 3 CH_3), 0.32 (s, 6H, 2 CH_3); ^{13}C NMR ($CDCl_3$) δ 146.0, 145.5, 134.8, 134.7, 130.9, 124.4, 124.2, 123.7, 123.4, 120.1, 118.8, 74.0, 39.8, 39.7, 39.4, 31.6, 26.8, 26.6, 25.8, 25.7, 23.8, 22.2, 20.9, 18.2, 17.6, 16.0, 15.9, 15.8, 11.9, −4.3; MS (EI) *m/z* 524 (M^+, 100%), 305 (13.7%), 265 (23.5%), 207 (15.6%), 167 (23.4%), 149 (63.7%), 69 (64.6%), 43 (48.8%).

Synthesis of (6E,10E)-3-bromo-13-[(R)-6-(tert-butyl-dimethyl-silanyloxy)-2,5,8-trimethyl-chroman-2-yl]-2,6,10-trimethyl-trideca-6,10-dien-2-ol ***(2b)***

To a solution of **1b** (670.0 mg, 1.28 mmol) in THF/water 7:1 (8 mL) at 0°C, NBS (250.0 mg, 1.40 mmol) was slowly added as a solid. The reaction mixture was stirred for 1.5 h and was then diluted with water for extraction with diethyl ether. After drying with anhydrous magnesium sulfate, the organic solution containing **2b** was concentrated under reduced pressure and remained crude for the next reaction. Yellow cloudy oil, R_f = 0.10 (DCM).

Synthesis of tert-butyl-{;(R)-2-[(3E,7E)-10-(3,3-dimethyl-oxiranyl)-4,8-dimethyl-deca-3,7-dienyl]-2,5,8-trimethyl-chroman-6-yloxy};-dimethyl-silane ***(3b)***

Crude **2b** was dissolved in a saturated solution of potassium carbonate in methanol (7 mL). An additional 2 mL DCM was added to solubilize **2b** and the mixture was allowed to stir at room temperature for 15 h. Later, it was extracted using DCM, dried with anhydrous magnesium sulfate, and concentrated to afford a crude epoxide **3b**. Yellow cloudy oil, R_f = 0.48 (Hexane/ethyl acetate 2:1).

Synthesis of (6E,10E)-13-[(R)-6-(tert-butyl-dimethyl-silanyloxy)-2,5,8-trimethyl-chroman-2-yl]-2,6,10-trimethyl-trideca-6,10-diene-2,3-diol ***(4b)***

600 mg of crude **3b** was dissolved in THF (1 mL) along with 3% $HClO_4$ (1 mL) and distilled water (750 μL). The reaction mixture was left stirring at room temperature for 4.5 h. It was then extracted with DCM, dried with anhydrous magnesium sulfate, and concentrated to yield crude **4b**. The diol was purified via column chromatography (Hexane/ethyl acetate 2:1). (235.2 mg, 0.42 mmol, 33% for three steps), clear yellow oil, R_f = 0.31 (Hexane/ethyl acetate 2:1), ^{1}H NMR ($CDCl_3$) δ 6.49 (s, 1H, Ar-H), 5.18 (overlapping triplets, 2H, C = CH), 3.36 (m, 1H, CHOH), 2.62 (t, 2H, C-4 H), 2.16 (m, 1H, C-3 H), 2.14–2.08 (m, 4H), 2.14 (s, 3H, Ar-CH_3), 2.08 (s, 3H, Ar-CH_3), 2.02 (m, 4H), 1.82 (m, 2H), 1.68-1.55 (m, 2H), 1.64 (s, 3H, CH_3), 1.62 (s, 3H, CH_3), 1.50–1.40 (m, 1H, C-3 H), 1.27 (s, 3H, C-2 CH_3), 1.22 (s, 3H, CH_3), 1.18 (s, 3H, CH_3), 1.04 (s, 9H, 3 CH_3), 0.20 (s, 6H, 2 CH_3); ^{13}C NMR ($CDCl_3$) 146.0, 145.5, 134.8, 124.9, 124.5, 123.8, 123.3, 120.1, 118.8, 78.2, 74.1, 73.0, 39.6, 39.3, 36.7, 31.5, 29.7, 26.5, 26.3, 25.8, 23.7, 23.2, 22.1, 20.9, 18.2, 16.0, 15.9, 15.8, 11.9, −4.3; MS (FAB) *m/z* 558 (M^+, 6.3%), 540 (6.5%), 524 (9.3%), 498 (9.6%), 208 (20.8%), 167 (31.3%), 84 (67.9%), 75 (100%), 57 (54.8%), 43 (82.4%).

Synthesis of (4E,8E)-11-[(R)-6-(tert-butyl-dimethyl-silanyloxy)-2,5,8-trimethyl-chroman-2-yl]-4,8-dimethyl-undeca-4,8-dienal ***(5b)***

4b (235 mg, 0.42 mmol) was dissolved in THF/water 2:1 (6 mL), then sodium periodate (179.7 mg, 0.84 mmol) was added. The reaction was monitored by TLC and went on for 15 h. It was extracted using DCM and dried with anhydrous magnesium sulfate. Aldehyde **5b** was not chromatographed and was used crude for the next step. Clear yellow oil, R_f = 0.66 (Hexane/ethyl acetate 2:1), MS (EI) *m/z* 498 (M^+, 4.4%), 362 (15.1%), 191 (51.3%), 111 (24.6%), 85 (52.6%), 71 (77.4%), 57 (100%), 43 (87.9%).

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Synthesis of tert-butyl-dimethyl-[(R)-2,5,8-trimethyl-2-((3E,7E)-13,13,13-trideutero-4,8-dimethyl-12-trideuteromethyl-trideca-3,7,11-trienyl)-chroman-6-yloxy]-silane ***(7b)***

6 (363.3 mg, 0.93 mmol) was dissolved in dry THF (10 mL) under an argon atmosphere and lithium bis(trimethylsilyl)amide (1.0 M in THF, 1.68 mL, 1.68 mmol) was added dropwise. The solution ylide was stirred for 1 h, then a solution of crude **5b** dissolved in dry THF (10 mL) was slowly added over the course of 40 min via cannula. After 15 h, the reaction mixture was quenched with ammonium chloride (10 mL) and **7b** was purified via column chromatography (DCM). (140.0 mg, 0.26 mmol, 62% for two steps), pale yellow oil, R_f = 0.65 (DCM), ^{1}H NMR ($CDCl_3$) δ 6.50 (s, 1H, Ar-H), 5.13 (overlapping triplets, 3H, C = CH), 2.62 (t, 2H, C-4 H), 2.16 (m, 1H, C-3 H), 2.16–2.06 (m, 4H), 2.14 (s, 3H, Ar-CH_3), 2.08 (s, 3H, Ar-CH_3), 2.01 (m, 4H), 1.82 (m, 2H), 1.72–1.53 (m, 2H), 1.62 (s, 6H, 2 CH_3), 1.28 (overlapping singlet and multiplet, 4H, C-2 CH_3, C-3 H), 1.04 (s, 9H, 3 CH_3), 0.21 (s, 6H, 2 CH_3); ^{2}H NMR (two drops of $CDCl_3$ in 600 μL $CHCl_3$) δ 1.63 (s, 3H, CD_3), 1.55 (s, 3H, CD_3); ^{13}C NMR ($CDCl_3$) δ 146.0, 145.5, 135.0, 134.9, 133.8, 133.6, 128.7, 128.5, 128.4, 124.4, 124.2, 123.8, 120.2, 118.8, 74.1, 39.7, 39.6, 31.5, 26.7, 26.6, 25.9, 23.8, 22.2, 20.9, 18.2, 16.0, 15.8, 11.9, −4.2; MS (EI) *m/z* 530 (M^+, 7.6%), 394 (11.5%), 277 (53.7%), 262 (100%), 183 (79.8%), 108 (43.6%), 71 (27.2%), 57 (52.1%), 43 (38.2%).

(R)-2,5,8-trimethyl-2-((3E,7E)-13,13,13-trideutero-4,8-dimethyl-12-trideuteromethyl-trideca-3,7,11-trienyl)-chroman-6-ol ***(8b)***

7b (140.0 mg, 0.26 mmol) was dissolved in dry THF (5 mL) and tetrabutylammonium fluoride (1.0 M in THF, 1.00 mL, 1.00 mmol) was added dropwise. The solution was stirred at room temperature for 2 h and then quenched with 1 N HCl (2.5 mL). Product **8b** was purified via column chromatography (DCM/hexane 3:1). (86.0 mg, 0.21 mmol, 81%), pale yellow oil, R_f = 0.36 (DCM/hexane, 3:1), 97.6% isotopically pure, ^{1}H NMR ($CDCl_3$) δ 6.51 (s, 1H, Ar-H), 5.15 (overlapping triplets, 3H, C = CH), 2.65 (t, 2H, C-4 H), 2.20–2.07 (m, 6H), 2.15 (s, 3H, Ar-CH_3), 2.13 (s, 3H, Ar-CH_3), 2.02 (m, 4H), 1.84 (m, 2H), 1.73–1.55 (m, 2H), 1.63 (s, 6H, 2 CH_3), 1.29 (s, 3H, C-2 CH_3); ^{13}C NMR ($CDCl_3$) δ 145.8, 145.7, 135.0, 134.9, 124.4, 124.3, 124.2, 124.0, 120.2, 119.2, 115.3, 74.2, 39.7, 39.6, 39.4, 31.4, 26.7, 26.6, 23.7, 22.2, 20.8, 16.0, 15.9, 15.8, 10.9; MS (EI) *m/z* 416 (M^+, 14.9%), 414 (42.6%), 280 (7.3%), 191 (17.3%), 167 (14.7%), 151 (51.5%), 75 (100%), 44 (52.4%).

Terminally Deuterated γ-Tocotrienol

Synthesis of tert-butyl-dimethyl-[(R)-2,7,8-trimethyl-2-((3E,7E)-4,8,12-trimethyl-trideca-3,7,11-trienyl)-chroman-6-yloxy]-silane ***(1c)***

γ-Tocotrienol (1.37 g, 3.33 mmol) and imidazole (900 mg, 13.2 mmol) were dissolved in 14 mL anhydrous DMF under an argon atmosphere. *tert*-Butyldimethylsilyl chloride (753 mg, 5.0 mmol) was added to the mixture and it was heated in an oil bath to 85°C overnight protected with a calcium chloride drying tube. The reaction mixture was then diluted with water and extracted using diethyl ether. The organic layer was dried with anhydrous sodium sulfate and concentrated under reduced pressure to afford a light yellow oil. Compound **1c** was purified via column chromatography (DCM). (1.70 g, 3.24 mmol, 97%), light yellow oil, R_f = 0.76 (DCM), ^{1}H NMR ($CDCl_3$) δ 6.36 (Ar-H), 5.15 (overlapping triplets, 3H, C = CH), 2.69 (t, 2H, C-4 H), 2.18 (m, 2H, C-2′ H), 2.11 (s, 6H, Ar-CH_3), 2.08 (m, 4H, CH_2), 1.99 (m, 4H, CH_2), 1.79,1.76 (m, 2H, C-3 H), 1.70 (s, 3H, CH_3), 1.62,1.57 (m, 2H, C-1 H), 1.61 (s, 9H, 3 CH_3), 1.27 (s, 3H, C-2 CH_3), 1.02 (s, 9H, 3 CH_3), 0.19 (s, 6H, 2 CH_3); ^{13}C NMR ($CDCl_3$) δ 146.08, 145.82, 135.05, 134.94, 131.23, 126.16, 125.64, 124.41, 124.22, 117.66, 115.78, 77.21, 75.14, 39.83, 39.71, 31.51, 26.76, 26.61, 25.87, 25.70, 24.03, 22.42, 22.22, 18.25, 18.13, 17.68, 16.00, 15.86, 12.84, 12.04, −4.24; MS (EI) *m/z* 524 (M^+, 27.8%), 265 (8.6%), 189 (43.7%), 147 (100%).

Synthesis of (6E,10E)-3-bromo-13-[(R)-6-(tert-butyl-dimethyl-silanyloxy)-2,7,8-trimethyl-chroman-2-yl]-2,6,10-trimethyl-trideca-6,10-dien-2-ol ***(2c)***

To a solution of **1c** (1.51 g, 2.88 mmol) in THF/water 7:1 (32 mL) at 0°C, NBS (565 mg, 3.17 mmol) was slowly added as a solid. The reaction mixture was stirred for 1 h and was then diluted with water for extraction with diethyl ether. After drying with anhydrous magnesium sulfate, the organic solution containing **2c** was concentrated under reduced pressure and remained crude for the next reaction. Yellow cloudy oil, R_f = 0.69 (DCM).

Synthesis of tert-butyl-{;(R)-2-[(3E,7E)-10-(3,3-dimethyl-oxiranyl)-4,8-dimethyl-deca-3,7-dienyl]-2,7,8-trimethyl-chroman-6-yloxyv};-dimethyl-silane ***(3c)***

Crude bromohydrin **2c** was dissolved in a saturated solution of potassium carbonate in methanol (28 mL). An additional 4 mL DCM was added to solubilize **2c** and the solution was allowed to stir at room temperature for 15 h. Afterward, it was extracted using DCM, dried with anhydrous magnesium sulfate, and concentrated to afford a crude epoxide **3c**. Yellow cloudy oil, R_f = 0.62 (DCM).

Synthesis of (6E,10E)-13-[(R)-6-(tert-butyl-dimethyl-silanyloxy)-2,7,8-trimethyl-chroman-2-yl]-2,6,10-trimethyl-trideca-6,10-diene-2,3-diol ***(4c)***

Crude **3c** was dissolved in THF (4 mL) along with 3% $HClO_4$ (4 mL) and distilled water (3 mL). The reaction mixture was left stirring at room temperature for 4.5 h. It was then extracted with DCM, dried with anhydrous magnesium sulfate, and concentrated to yield crude **4c**. The diol was purified via column chromatography (Hexane/ethyl acetate 2:1). (399 mg, 0.72 mmol, 25% for three steps), clear yellow oil, R_f = 0.32 (Hexane/ethyl acetate 2:1), ^{1}H NMR ($CDCl_3$) δ 6.36 (Ar-H), 5.18 (m, 2H, C = CH), 3.38 (m, 1H, CH_2OH), 3.35 (m, 1H, CH_2OH), 2.69 (m, 2H, C-4 H), 2.18 (m, 2H, C-2′ H), 2.11 (s, 6H, Ar-CH_3), 2.06 (m, 4H, CH_2), 2.00 (m, 4H, CH_2), 1.79,1.76 (m, 2H, C-3 H), 1.63 (s, 3H, CH_3), 1.62,1.57 (m, 2H, C-1 H), 1.61 (s, 3H, CH_3), 1.27 (s, 3H, C-2 CH_3), 1.21 (s, 3H, 3 CH_3), 1.17 (s, 3H, 3 CH_3), 1.02 (s, 9H, 3 CH_3), 0.19 (s, 6H, 2 CH_3); ^{13}C NMR ($CDCl_3$) δ 146.09, 145.81, 134.88, 126.16, 125.62, 125.05, 124.56, 117.66, 115.78, 78.31, 77.20, 75.12, 72.96, 39.83, 39.60, 36.78, 31.50, 29.64, 26.51, 26.42, 25.86, 24.01, 23.31, 22.41, 22.21, 18.25, 15.90, 15.82, 12.84, 12.04, −4.25; MS (EI) *m/z* 558 (M^+, 2.9%), 524 (33.3%), 265 (29.4%), 75 (100%).

Synthesis of (4E,8E)-11-[(R)-6-(tert-butyl-dimethyl-silanyloxy)-2,7,8-trimethyl-chroman-2-yl]-4,8-dimethyl-undeca-4,8-dienal ***(5c)***

4c (376 mg, 0.67 mmol) was dissolved in THF/water 2:1 (15 mL), then sodium periodate (290 mg, 1.36 mmol) was added. The reaction was monitored by TLC and went on for 15 h. It was extracted using DCM and dried with anhydrous magnesium

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sulfate. Some of aldehyde **5c** was chromatographed (DCM) for identification, and the remainder was used crude in the next step. Clear yellow oil, $R_f = 0.68$ (DCM), ^{1}H NMR ($CDCl_3$) δ 9.75 (t, 1H, C<u>H</u>O, $J = 1.85$ Hz), 6.38 (s, 1H, Ar-H), 5.16 (overlapping triplets, 2H, C = C<u>H</u>), 2.70 (t, 2H, C-4 <u>H</u>), 2.52 (t, 2H), 2.33 (t, 2H), 2.18 (m, 1H, C-3 <u>H</u>), 2.13 (overlapping singlets, 6H, Ar-C<u>H</u>$_3$), 2.08 (m, 4H), 1.80 (m, 4H), 1.63 (s, 3H, C<u>H</u>$_3$), 1.61 (s, 3H, C<u>H</u>$_3$), 1.45 (s, 1H, C-3 <u>H</u>),1.30 (s, 3H, C-2 C<u>H</u>$_3$), 1.04 (s, 9H, 3 C<u>H</u>$_3$), 0.21 (s, 6H, 2 C<u>H</u>$_3$); ^{13}C NMR ($CDCl_3$) δ 202.57, 146.10, 145.82, 134.68, 132.91, 126.15, 125.61, 125.36, 124.67, 117.66, 115.81, 75.11, 42.15, 39.78, 39.47, 31.85, 31.53, 26.48, 25.89, 24.07, 22.42, 22.22, 18.26, 16.09, 15.83, 12.87, 12.07, −4.22; MS (EI) *m/z* 498 (M^+, 99.3%), 362 (60.2%), 265 (267.0%), 73 (100%).

Synthesis of tert-butyl-dimethyl-[(R)-2,7,8-trimethyl-2-((3E,7E)-13,13,13-trideutero-4,8-dimethyl-12-trideuteromethyl-trideca-3,7,11-trienyl)-chroman-6-yloxy]-silane ***(7c)***

Phosphonium salt **6** (207 mg, 0.53 mmol) was dissolved in dry THF (10 mL) under an argon atmosphere and lithium bis(trimethylsilyl)amide (1.0 M in THF, 1.3 mL, 1.3 mmol) was added dropwise. The solution of ylide was stirred for 1 h, then a solution of crude aldehyde **5c** (228 mg, 0.46 mmol) dissolved in dry THF (5 mL) was slowly added via cannula. After 15 h, the reaction mixture was quenched with ammonium chloride (10 mL) and **7c** was purified via column chromatography (DCM) (99.7 mg, 0.19 mmol, 36% for two steps), pale yellow oil, $R_f = 0.82$ (DCM), ^{1}H NMR ($CDCl_3$) δ 6.39 (Ar-<u>H</u>), 5.16 (overlapping triplets, 3H, C = C<u>H</u>), 2.72 (t, 2H, C-4 <u>H</u>), 2.18 (m, 2H, C-2′ <u>H</u>), 2.14 (s, 6H, Ar-C<u>H</u>$_3$), 2.10 (m, 4H, C<u>H</u>$_2$), 2.04 (m, 4H, C<u>H</u>$_2$), 1.81 (m, 2H, C<u>H</u>$_2$), 1.79,1.76 (m, 2H, C-3 <u>H</u>), 1.70 (s, 3H, C<u>H</u>$_3$), 1.62,1.57 (m, 2H, C-1 <u>H</u>), 1.64 (s, 6H, 2 C<u>H</u>$_3$), 1.30 (s, 3H, C-2 C<u>H</u>$_3$), 1.05 (s, 9H, 3 C<u>H</u>$_3$), 0.22 (s, 6H, 2 C<u>H</u>$_3$); ^{13}C NMR ($CDCl_3$) δ 146.10, 145.84, 135.05, 134.95, 131.04, 126.17, 125.65, 125.53, 124.42, 124.23, 117.66, 115.79, 75.14, 39.85, 39.75, 39.72, 31.53, 30.34, 29.73, 26.76, 26.62, 25.89, 24.05, 22.44, 22.24, 18.27, 16.02, 15.88, 12.86, 12.05, -4.24; MS (EI) *m/z* 530 (M^+, 28.0%), 394 (82.0%), 262 (84.3%), 183 (67.8%), 43 (100%).

(R)-2,7,8-trimethyl-2-((3E,7E)-13,13,13-trideutero-4,8-dimethyl-12-trideuteromethyl-trideca-3,7,11-trienyl)-chroman-6-ol ***(8c)***

7c (176 mg, 0.33 mmol) was dissolved in dry THF (5 mL) and tetrabutylammonium fluoride (1.0 M in THF, 1.7 mL, 1.7 mmol) was added dropwise. The solution was stirred at room temperature for 2 h and then quenched with 1 N HCl (2.5 mL). Product **8c** was purified via column chromatography (DCM/hexane 3:1). Pale yellow oil (101 mg, 0.24 mmol, 73%), $R_f = 0.25$ (DCM/hexane, 3:1); ^{1}H NMR ($CDCl_3$) δ 6.39 (Ar-<u>H</u>), 5.11 (overlapping triplets, 3H, C = C<u>H</u>), 2.72 (t, 2H, C-4 <u>H</u>), 2.18 (m, 2H, C-2′ <u>H</u>), 2.15 (s, 3H, Ar-C<u>H</u>$_3$), 2.14 (s, 3H, Ar-C<u>H</u>$_3$), 2.10 (m, 4H, C<u>H</u>$_2$), 2.01 (m, 4H, C<u>H</u>$_2$), 1.79,1.76 (m, 2H, C-3 C<u>H</u>$_2$), 1.62 (s, 3H, C<u>H</u>$_3$), 1.61(s, 3H, C<u>H</u>$_3$), 1.65,1.59 (m, 2H, C-1 C<u>H</u>$_2$), 1.28 (s, 3H, C-2 C<u>H</u>$_3$); ^{13}C NMR ($CDCl_3$) δ 146.27, 145.70, 135.08, 134.97, 125.81, 124.43, 124.35, 124.19, 121.62, 118.24, 112.13, 77.21, 75.22, 39.79, 39.72, 39.69, 31.41, 29.42, 26.74, 26.60, 25.61, 23.99, 22.28, 22.21, 15.99, 15.88, 11.89, 11.85; MS (EI) *m/z* 416 (M^+, 46.4%), 280 (13.7%), 165 (39.1%), 151 (100%), 75 (70.0%).

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