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Research Article

5-Trideuteromethyl-α-tocotrienol and 5-$^{14}CH_3$-α-tocotrienol as biological tracers of tocotrienols

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Summary

The tocotrienols have attracted increased attention recently as evidence has accrued that their biological activities are significantly different from tocopherols. The biokinetics and metabolic fate of tocopherols have long been studied using deuteromethylated forms of α-tocopherol prepared by a stannous chloride catalysed paraformaldehyde methylation of γ- and δ-tocopherols. We show here that his methodology is not an efficient route to deuterated α-tocotrienol because of low yields and extensive exchange of allylic hydrogens under the prolonged acidic conditions of the deuteromethylation. Instead, we have prepared deuteromethylated and ^{14}C-radiolabelled α-tocotrienol by aminomethylation at C-5 of γ-tocotrienol (available from palm oil), followed by reduction with $NaCNBD_3$ in refluxing iso-butanol. The deuteromethylation procedure is amenable to multi-gram scale reactions.

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Key Words: tocotrienol; deuteration; aminomethylation

Introduction

The family of compounds collectively known as vitamin E are made up of the tocopherols that have a saturated side chain derived from phytol, and the tocotrienols that have three sites of unsaturation in the geranylgeranyl

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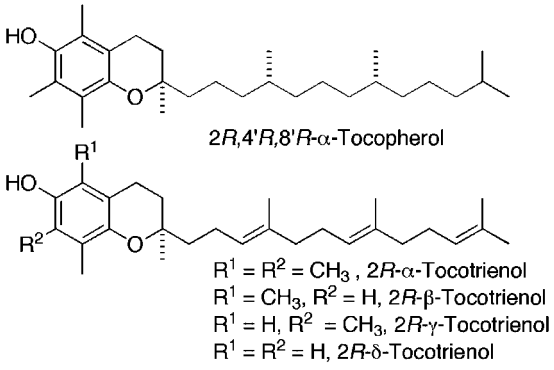

Figure 1.

diphosphate-derived[1] C_{16} side chain. Figure 1 shows the structures of the naturally occurring stereoisomers of α-tocopherol and the tocotrienols.

The centrality of α-tocopherol to mammalian metabolism has been established by extensive biokinetic studies detailing the specificity of α-tocopherol retention over other forms of vitamin E mediated by the α-tocopherol transfer protein (α-TTP). These studies have been aided by the use of tocopherols that have deuteromethyl groups at positions on the chroman ring.[2,3] Following administration of deuterated tocopherols to animals, plasma and tissues can be extracted and the quantities of each labelled and non-labelled tocopherol analysed by GC-MS. Thus, 5-CD_3-α-tocopheryl acetate and 5,7-$(CD_3)_2$-α-tocopherol have been used to follow the absorption and fate of the acetate and free phenol forms of α-tocopherol.[4, 5] Similarly, the stereoselectivity of absorption and distribution of tocopherol has been followed using deuterated RRR- and SRR-forms of α-tocopherol.[6, 7]

The tocotrienols have been demonstrated to have significantly different biological activities from the tocopherols[8] including inhibition of cholesterol biosynthesis[9–11], anti-cancer effects[12–14], and more recently protection against glutamate-induced neurodegeneration.[15–17] Studies on the mechanism of these effects would benefit from traceable forms of tocotrienol for use in cell culture and animal experiments. We report here a straightforward method for the synthesis of 5-CD_3- and 5-$^{14}CH_3$-α-tocotrienol.

Results

Natural source γ-tocotrienol was the starting material for the reactions reported here. It is the major tocotrienol found in palm kernel oil concentrates such as TOCOMIN®-50 from which about 270 mg can be isolated from 2.0 g of the mixture. α- and δ-Tocotrienol could also be isolated in quantities of about 210 and 110 mg, respectively, from the same 2.0 g mixture. The identity

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and purity of the isolated tocotrienols were confirmed by ^{1}H-NMR, ^{13}C-NMR and mass spectroscopy.

Methylation of tocopherols has normally been accomplished using paraformaldehyde and $SnCl_2$ as a Lewis acid catalyst in a mixture of HCl and diisopropyl ether at reflux.[2,3,18] We attempted this method to see how it would perform on a tocotrienol and were not surprised that yields were rather low (27%) given the likely sensitivity of the trisubstituted alkenes in the acidic reaction conditions. Furthermore, even during brief reaction times, more than three deuterium atoms were found in the isolated product, presumably due to allylic H/D exchange occurring above the expected incorporation of the trideuteromethyl group. After 5/h of reflux in diisoproyl ether (chemical yields 27%) the deuterium content was d_3 (92.1%), d_4 (3.7%), and d_5 (4.2%) as judged by the isotopic intensities of the molecular ion from mass spectrometry. Prolonged refluxing for 6 days did not increase the yield (in fact it diminished to 18%) and the product was now found to have incorporated a large number of deuteriums with a median abundance of d_{16} spanning from approximately d_6 to d_{21}. The degree of deuterium incorporation at specific sites was not explored.

Industrial methods for transforming mixed tocols to α-tocopherol avoid the use of transition metals in the alkylation step and instead use either a Mannich or hydroxymethylation reagent to prepare intermediate amino or hydroxymethylated tocols that are then hydrogenated to reduce the benzylic C–N or C–O bond.[19–23] Catalytic hydrogenation is inappropriate with the tocotrienols as the unsaturated side chain would also be reduced. We therefore explored whether the intermediate amino/hydroxymethyl tocols could be reduced with hydride reagents that would leave the side chain double bonds untouched. We have found that $NaCNBD_3$ efficiently reduced the aminomethylated compounds (such as **1**) in refluxing *iso*-butanol (bp. 108°C).[19] Preliminary reactions were performed on 50–100 mg scale, but worked equally well on a 2–5 g scale. The deuterium incorporation in **2** was; d_3 (98.0%), d_4 (1.5%), and d_5 (0.5%).

As there is also some need for deuterated forms of γ-tocotrienol, given its notably different biological profile to α-tocotrienol,[11,24–26] we wondered whether the aminomethylation of δ-tocotrienol might deliver useful amounts of γ-tocotrienol besides β-tocotrienol, which was expected to be formed as the major regioisomer. Unfortunately, treating δ-tocotrienol with the Mannich reagent used in Figure 2 yielded only 5-CD_3-β-tocotrienol, **3**, in 52% isolated yield after reduction. This result is in agreement with the observation that aminomethylation of δ-tocopherol yields almost exclusively the 5-aminomethyl intermediate under somewhat milder reaction conditions (80°C).[19] The regiochemistry is substantiated by the observation that a nuclear Overhauser effect (nOe) was evident when the single aromatic proton in the H-NMR of the

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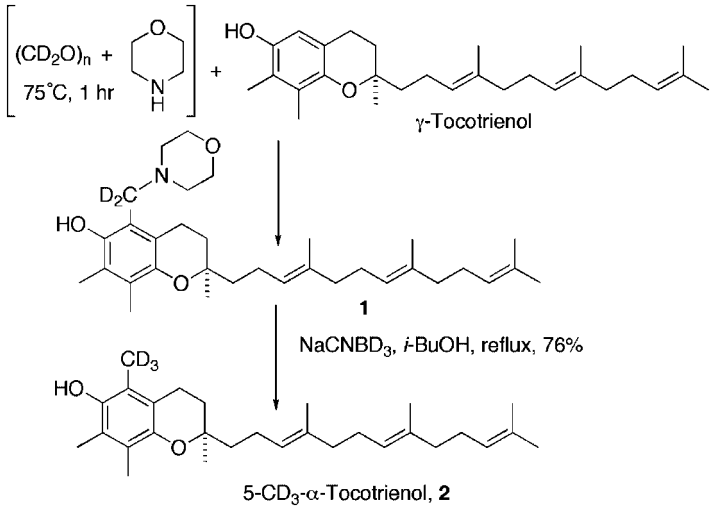

Figure 2.

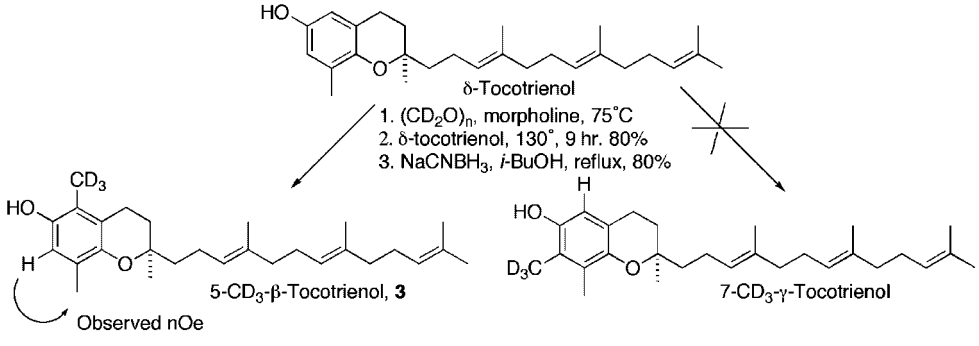

Figure 3.

product was irradiated and the resonance for the C-8 methyl group enhanced. No such enhancement would be predicted to occur if 7-CD_3-γ-tocotrienol had been the product (Figure 3).

We also attempted to prepare α-tocotrienol chromene by oxidation of α-tocotrienol with dichlorodicyanoquinone (DDQ) with the hope that the chromene **4** could be selectively reduced using diimide generated from potassium azodicarboxylate and deuterated acetic acid (CH_3COOD).[27] This would generate a dideuterated compound **5** similar to the d_2-γ-tocopherol we prepared earlier.[28] While DDQ oxidation of α-tocopheryl acetate proceeds well in refluxing toluene,[18,28,29] similar conditions failed to oxidize α-tocotrienyl acetate. The use of higher boiling xylenes with DDQ lead to decomposition of the tocotrienol. It is possible to prepare tocotrienol

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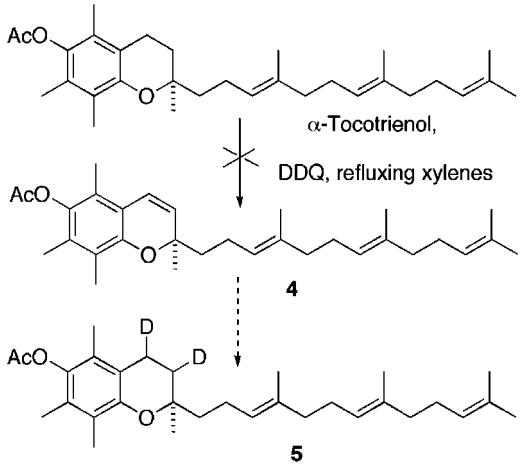
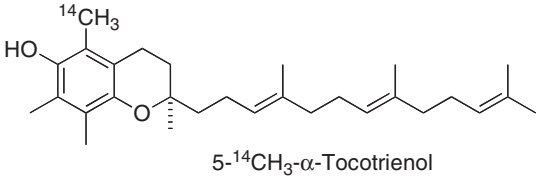


Figure 4.

chromene by condensing 2-acetyl-3,5,6-trimethylhydroquinone with farnesylacetone,[30] but this prepares material that is racemic and we desired only the natural R-stereochemistry (Figure 4).

The method outlined in Figure 2 was also used to make a ^{14}C-labelled α-tocotrienol by substituting ^{14}C-paraformaldehyde. Trial reactions on a small scale (~3 mg unlabelled paraformaldehyde) gave the best yield (60%) when a five-fold excess of γ-tocotrienol was used. The unreacted γ-tocotrienol could easily be removed from the crude product by preparative TLC (hexane:EtOAc 9:1). The radiochemical reaction on a similar scale gave a lesser yield (29% after purification), but still provided 12.4 mg of α-tocotrienol having specific activity of 0.7 mCi/mmol. The material was spectroscopically indistinguishable from standard α-tocotrienol as the content of ^{14}C at this specific activity is only 0.38%.



Conclusion

We have described here a convenient method, useful at a gram scale, for the preparation of deuterated and ^{14}C-radiolabelled α-tocotrienol starting from the naturally occurring γ-tocotrienol. Their use will enable biokinetic and metabolic studies of this vitamin in cell culture and animal trials.

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Experimental

All reagents were purchased from Aldrich Chemical Company, Oakville, ON; $NaCNBD_3$ from CDN Isotopes. Inc. Pointe-Claire, Quebec; TOCOMIN 50® from Carotech Sdn Bhd. Malaysia and ^{14}C-labelled paraformaldehyde from American Radiolabeled Chemical Inc., St. Louis, MO. All glassware and syringes were dried in an oven at 60–80°C, and then cooled in a dry box before use. A constant temperature silicon oil bath was used for heating reaction mixtures at temperatures above room temperature.

Reagent-grade solvents (Caledon Laboratories, Georgetown, Ontario) were used for all extractions and work-up procedures. Tetrahydrofuran (THF) and benzene were distilled from sodium benzophenone ketyl. Dichloromethane and hexane were distilled from CaH_2.

Thin layer chromatography (TLC) was carried out on Merck pre-coated silica gel 60 F_{254}, aluminium sheets, 200 μm thickness, 25 mm (width) × 50 mm (length). After development, the sheets were viewed under short wavelength UV light (254 nm) or with an oxidizing staining solution consisting of 4% sulphuric acid in methanol, followed by heating using a hot air gun. Preparative TLC used Uniplate™ silica gel GF plates (2000 μm) from Analtech (Newark, Delaware). Flash chromatography was performed using Merck 9385 silica gel 60 (230–400 mesh). ^1H(300 MHz) and ^{13}C NMR (75 MHz) spectra were obtained on a Bruker DPX-300 digital FT NMR spectrometer with deuterated chloroform as solvent. Chemical shifts for NMR were determined relative to the internal standard tetramethylsilane (δ 0.00 ppm) or $CHCl_3$ (δ 7.24 ppm) for ^1H spectra, and $CDCl_3$ (δ 77.0 ppm) for ^{13}C spectra. 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 introduced through a direct inlet system. Ions were generated using electron impact (EI) at 70 eV.

Separation and purification of tocotrienols from commercial palm oil concentrates

TOCOMIN®50 contains ~ 50% (w/w) natural tocotrienols and tocopherols, which consist of predominantly α-, β-, γ-, δ-tocotrienols and α-tocopherol. Column chromatography using 10% EtOAc in hexane was used to separate and purify these ingredients for use as starting materials in the syntheses of deuterated and radiolabelled tocotrienols. From 2.0 g of TOCOMIN®50 it was possible to retrieive ~260 mg of γ-tocotrienol, 200 mg of α-tocotrienol, and 110 mg of δ-tocotrienol.

α-Tocotrienol TLC: R_f = 0.45 (hexane/EtOAc = 9:1) ^1H-NMR ($CDCl_3$) 5.18 (t, 1 H, vinyl) 5.15 (overlapping triplets, 2 H, vinyl), 4.21 (s, 1 H, OH), 2.62

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(t, 2 H, $J = 6.8$ Hz, C4-CH_2), 2.14 (s, 3 H, Ar- CH_3), 2.13 (m, hidden under Ar-CH_3 peaks, 2 H, C2′-CH_2), 2.12 (s, 3 H, Ar- CH_3), 2.11 (s, 3 H, Ar- CH_3), 2.06 (m, 4 H CH_2), 1.97 (m, 4 H, CH_2), 1.81 and 1.77 (doublet of pentets, 2 H, $J_1 = 35.5$ Hz, $J_2 = 6.8$ Hz, C3-CH_2), 1.68 (s, 3 H, CH_3), 1.65 and 1.55 (m, 1 H each), 1.62 (s, 6 H), 1.60 (s, 3 H, CH_3), 1.23 (s, 3 H, C2-CH_3). ^{13}C-NMR ($CDCl_3$) 145.63, 144.71, 135.17, 135.08, 131.37, 124.55, 124.35, 122.76, 121.18, 118.63, 117.43, 74.42, 39.85, 39.67, 31.72, 26.90, 26.74, 25.83, 23.86, 22.37, 20.88, 17.82, 16.13, 16.03, 12.34, 11.91, 11.40. MS[EI+](%) *m/z* 424 (M^+, 71.1), 205 (15.1), 165 (100), 69 (47.9). HRMS (EI): Calculated for $C_{29}H_{44}O_2$ 424.33413; found: 424.33455.

γ-Tocotrienol TLC. $R_f = 0.32$ (hexane/EtOAc = 9:1) ^{1}H-NMR ($CDCl_3$) 6.37 (s, 1 H, Ar-H), 5.12 (m, 3 H, C3′H, C7′H, C11′H), 4.46 (br, 1 H, OH), 2.68 (m, 2 H, C4-CH_2), 2.18 (m, 2 H, C2'-CH_2), 2.15 (s, 3 H, Ar-CH_3), 2.13 (s, 3 H, Ar-CH_3), 2.08 (m, 4 H, 2CH_2), 2.00 (m, 4 H, 2CH_2), 1.80,1.74 (m, 2 H, C3-CH_2), 1.69 (s, 3 H, CH_3), 1.65, 1.58 (m, 1 H), 1.62 (s, 6 H, C12′-2CH_3), 1.60 (s, 3 H, CH_3), 1.26 (s, 3 H, C2-CH_3). ^{13}C-NMR ($CDCl_3$) 146.44, 145.78, 135.19, 135.07, 131.36, 125.91, 124.54, 124.48, 124.33, 121.81, 118.32, 112.29, 75.34, 39.91, 39.84, 31.66, 26.89, 26.72, 25.82, 24.12, 22.41, 22.34, 17.81, 16.12, 16.01, 12.02, 11.99. MS [EI+](%) *m/z* 410 (M^+, 59.8), 191 (24.3), 151 (100), 69 (73.1). HRMS (EI): Calculated for $C_{28}H_{42}O_2$ 410.31848; found: 410.31697.

δ-Tocotrienol TLC. $R_f = 0.25$ (hexane/EtOAc = 9:1) ^{1}H-NMR ($CDCl_3$) 6.33 (d, 1 H, $J = 3$ Hz, Ar-H), 6.22 (d, 1 H, $J = 3$ Hz, Ar-H), 4.96 (m, 3 H, C3′-H, C7′-H, C11′-H), 2.53 (m, 2 H, C4-CH_2), 1.98 (s, 3 H, Ar-CH_3), 1.87 (m, 10 H, CH_2), 1.65 (m, 2 H, C3-CH_2), 1.52 (s, 3 H, CH_3), 1.42 (s, 9 H, 3 CH_3), 1.42 (m, 2 H, C1′-CH_2), 1.11(s, 3 H, C2-CH_3). ^{13}C-NMR ($CDCl_3$) 148.18, 146.31, 135.52, 135.36, 131.66, 127.73, 124.79, 124.67, 121.60, 116.03, 112.97, 77.62, 75.69, 40.10, 40.07, 40.06, 30.71, 26.97, 26.85, 26.10, 24.42, 23.06, 22.39, 18.09, 16.46, 16.40, 16.27. MS [EI+](%) *m/z* 396 (M+, 47.3), 192 (13.5), 177 (31.4), 137 (75.8), 69 (100). HRMS (EI): Calculated for $C_{27}H_{40}O_2$ 396.30283; found: 396.30263.

Aminomethylation of γ-tocotrienol to give (R)-2,7,8-trimethyl-5-morpholin-4-yl-[$^2H_2$]-methyl-2-((3E,7E)-4,8,12-trimethyl-trideca-3,7,11-trienyl)-chroman-6-ol, **1**

Morpholine (500 μL, 5.43 mmol) and $(CD_2O)_n$ (94.3 mg, 2.94 mmol) were mixed in a round bottom flask and heated at 75°C in oil bath for 1 h. γ-Tocotrienol (972 mg, 2.37 mmol) was added to the mixture and the temperature was increased to 130°C. The reaction was stirred at 130°C for 9 h. TLC (hexane: EtOAc/ 9:1) showed that no starting material was left. Column chromatography with the same solvent system gave **1** (968.3 mg, 80%). TLC $R_f = 0.19$ (hexane/EtOAc = 9:1) ^{1}H-NMR ($CDCl_3$) 5.12 (m, 3H, C3′-H, C7′-H, C11′-H), 3.78 (br, 4H, morpholine-*O*-CH_2), 2.63 (m, 4H,

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morpholine-*N*-CH_2), 2.63 (m, 2H, C4-CH_2), 2.17 (s, 3H, Ar- CH_3), 2.14 (s, 3H, Ar- CH_3), 2.09–2.2 (m, 10H, CH_2), 1.81 (m, 2H, C3-CH_2), 1.71 (s, 3H, CH_3), 1.61 (s, 9H, $3CH_3$), 1.50–1.75 (m, 2H, CH_2), 1.26 (s, 3H, CH_3). ^{13}C-NMR ($CDCl_3$) 148.92, 144.74, 135.49, 135.34, 131.64, 125.64, 124.77, 124.72, 124.56, 123.12, 116.40, 114.74, 77.64, 74.48, 67.17, 53.12, 40.10, 40.08, 31.93, 27.13, 26.97, 26.11, 24.01, 22.58, 20.85, 18.09, 16.40, 16.27, 12.29, 12.15 MS [EI+] *m/z* 430 (100%), 205 (10%), 165 (90%).

(R)-2,7,8-trimethyl-5-[$^2H_3$]-methyl-2-((3E,7E)-4,8,12-trimethyl-trideca-3,7, 11-trienyl)-chroman-6-ol (5-CD_3-α-tocotrienol), **2**

1 (968.3 mg, 1.89 mmol) was mixed with $NaCNBD_3$ (563.9 mg, 8.56 mmol) in *i*-BuOH 15 mL. The reaction mixture was heated to reflux for 6.5 h, and then quenched with 2 M HCl until no gas evolution was evident. The mixture was extracted with Et_2O (3 × 10 ml). The ethyl ether layer was washed with saturated $NaHCO_3$ solution, dried over $MgSO_4$ and concentrated *in vacuo*. Column chromatography using hexane: EtOAc/ 9:1 gave **2** as yellow oil (613 mg, 76%). TLC R_f = 0.45 (hexane/EtOAc = 9:1) ^{1}H-NMR ($CDCl_3$) 5.14 (m, 3H, C3′-H, C7′-H, C11′-H), 4.25 (s, 1H, OH), 2.63 (t, 2H, J = 7 Hz, C4-CH_2), 2.17 (s, 3H, Ar- CH_3), 2.13 (s, 3H, Ar- CH_3), 2.13 (m, 2H, C2′-CH_2), 2.12 (s, 3H, Ar- CH_3), 2.10 (m, 4H CH_2), 1.99 (m, 4H, CH_2), 1.80 (m, 2H, C3-CH_2), 1.68 (s, 3H, CH_3), 1.65, 1.55 (m, 1H of each, C1′-CH_2), 1.62 (m, 6H, C12′-$2CH_3$), 1.60 (s, 3H, CH_3), 1.26 (s, 3H, C2-CH_3). ^{13}C-NMR ($CDCl_3$) 145.88, 145.00, 135.44, 135.35, 131.66, 124.81, 124.61, 123.02, 121.45, 118.82, 117.72, 77.64, 74.68, 40.12, 40.10, 39.91, 32.01, 27.15, 26.99, 26.11, 24.12, 23.08, 21.45, 18.09, 16.40, 16.30, 14.54, 12.61, 12.19. MS [EI+](%) *m/z* 427(M^+, 98.8), 191 (23.2), 168 (80.9), 69 (100). HRMS (EI): Calculated for $C_{29}H_{41}D_3O_2$ 427.35296; found: 427.35204.

(R)-2,8-dimethyl-5-[$^2H_3$]-methyl-2-((3E,7E)-4,8,12-trimethyl-trideca-3,7,11-trienyl)-chroman-6-ol, (5-CD_3-β-tocotrienol), **3**

Morpholine (100 µl, 1.26 mmol) and paraformaldehyde (4 mg, 0.125 mmol) were mixed in a round bottom flask and heated at 80°C in oil bath for 1 h. δ-Tocotrienol (49.3 mg, 0.12 mmol) was added to the mixture and the temperature was increased to 130°C. The reaction mixture was stirred at 130°C for 16 h at which time TLC (hexane: EtOAc, 9:1) showed no starting material remained. Silica gel column chromatography with the same solvent system gave the (*R*)-2,8-dimethyl-5-morpholin-4-yl-[$^2H_2$]-methyl-2-((3*E*,7*E*)-4,8,12-trimethyl-trideca-3,7,11-trienyl)-chroman-6-ol (39.9 mg, 65%). TLC R_f = 0.15 (hexane/EtOAc = 9:1) ^{1}H-NMR ($CDCl_3$) 6.56 (s, 1H, Ar-H), 5.12 (m, 3H, C3′-H, C7′-H, C11′-H), 3.78(br, 4H, morpholine-*O*-CH_2), 2.63 (m, 6H, 4H from morpholine-*N*-CH_2; 2H from C4-CH_2), 2.14 (s, 3H, Ar-CH_3),

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2.09–2.2 (m, 10H, CH_2), 1.81 (m, 2H, C3-CH_2), 1.71 (s, 3H, CH_3), 1.61 (s, 9H, $3CH_3$), 1.50–1.75 (m, 2H, C1′-CH_2), 1.26 (s, 3H, C2-CH_3). ^{13}C-NMR ($CDCl_3$) 150.39, 145.26, 135.48, 135.34, 131.63, 127.31, 121.41, 124.98, 124.77, 124.55, 119.15, 116.96, 115.38, 77.65, 75.68, 74.52, 67.15, 53.22, 53.14, 40.10, 39.81, 31.83, 27.13, 26.96, 26.11, 24.05, 22.85, 20.97, 18.09, 16.51, 16.40, 16.26, 11.91. MS [EI+](%) *m/z* 424 (73), 410 (68), 165 (97), 69 (100).

The product from the above reaction (39.9 mg, 0.08 mmol) was mixed with $NaCNBD_3$ (26.3 mg, 0.4 mmol) in 1 ml of i-BuOH. The suspension was heated to reflux for 6 h then quenched with drops of 2 M HCl until no gas evolution was visible. The mixture was extracted with Et_2O (3×10 ml), the ether layer washed with saturated $NaHCO_3$ solution, dried over anhydrous $MgSO_4$ and then concentrated *in vacuo*. Column chromatography using hexane: EtOAc (9:1) gave **3** as yellow oil (17.2 mg, 52%). TLC $R_f = 0.32$ (hexane/EtOAc = 9:1) ^{1}H-NMR ($CDCl_3$) 6.46 (s, 1H, Ar-H), 5.08 (m, 3H, C3′-H, C7′-H, C11′-H), 2.59 (t, 2H, $J = 7$ Hz, C4-CH_2), 2.10 (s, 3H, Ar- CH_3), 2.00 (m, 10H CH_2), 1.80 (m, 2H, C3-CH_2), 1.68 (s, 3H, CH_3), 1.65 (m, 2H, C1′-CH_2), 1.62 (m, 6H, $2CH_3$), 1.55 (m, 3H, CH_3), 1.30 (s, 3H, C2-CH_3). ^{13}C-NMR ($CDCl_3$) 146.29, 146.12, 135.48, 135.35, 131.66, 124.78, 124.73, 124.57, 124.46, 120.69, 119.46, 115.67, 74.64, 40.07, 39.77, 31.81, 27.13, 26.97, 26.09, 24.15, 22.56, 21.16, 18.08, 16.38, 16.24. MS [EI+](%) *m/z* 413 (M^+, 70), 194 (24), 154 (90), 69 (100). HRMS (EI): Calculated for $C_{28}H_{39}D_3O_2$ 413.33731; found: 413.33668.

5-$^{14}CH_3$-α-tocotrienol

[^{14}C]-Paraformaldehyde (~760 μCi, 1.9 Ci/g, ~0.4 mg) and non-radiolabelled paraformaldehyde (2.6 mg, 0.087 mmol) were combined in a 2 ml vial. Morpholine (30 μl, 0.34 mmol) was added and the contents heated at 75°C in an oil bath for 1.5 h. γ-Tocotrienol (200 mg, 0.487 mmol) was added and the temperature was increased to 130° and stirred for 4.5 h. After cooling to room temperature the crude reaction product was purified by column chromatography with hexane: EtOAc/ 4:1 to give the ^{14}C-labelled 5-*N*-morpholinomethyl-α-tocotrienol. Relevant chromatographic fractions were combined, the solvent evaporated in a 5 ml thick-walled V-bottomed vial and replaced with 0.6 ml of *i*-BuOH, followed by addition of $NaCNBH_3$ (31.4 mg, 0.5 mmol). The vial was sealed tightly and heated at 110°C for 5.5 h. The reaction was quenched with 10% HCl until gas evolution ceased, then extracted with Et_2O (2.5 ml × 3). The ethyl ether layer was neutralized with saturated $NaHCO_3$ solution, dried over $MgSO_4$ and concentrated by purging with a stream of N_2. Preparative TLC chromatography using hexane: EtOAc/ 9:1 gave 5-^{14}C-methyl-α-tocotrienol as a light yellow oil with an overall yield of 29% (12.4 mg, 205 μCi) from the starting $(CH_2O)_n + (^{14}CH_2O)_n$. The specific activity was 0.7 mCi/mmol.

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