First Synthesis of (8-$^2H_3$)-(all-*rac*)-δ-Tocopherol

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Trideuterated tocopherols were needed to be used as internal standards for our study about simultaneous quantitative determination of tocopherols in foodstuffs by mass spectrometry. Whilst procedures for trideuterated α-tocopherol have been recently optimized, no method is reported as regards δ-tocopherol. Different synthetic approaches are discussed, as well as the first procedure for the synthesis of (8-$^2H_3$)-(all-*rac*)-δ-tocopherol.

Introduction

In our studies on the development of quantitative mass spectrometric determinations of compounds showing relevant nutritional properties in foodstuffs we focused our attention on tocopherols, the main components of the pool of compounds called vitamin E.

Vitamin E is protective against many diseases, such as cancer, cardiovascular diseases,[1] cell membrane and DNA damage by free radicals, oxidation of low-density lipoproteins[2] and disorders of other lipid-rich body constituents.[3] Tocopherols are present in oil seeds, leaves and other green parts of higher plants. Since vitamin E is only synthesized by plants, it is a very important dietary nutrient for humans and animals.[4] The tocopherol content in foods is also important to protect food lipids against autoxidation and thereby to increase their shelf life and value as wholesome foods.

The preferred separation methods for the determination of tocopherols are reversed and normal-phase HPLC[5] with UV and fluorimetric detection, the latter one being the most sensitive and accurate for quantifying trace amounts. In addition, there are other methods like GC[6] and capillary electrophoresis (CE).[7] Generally, the total tocopherol content is determined, even through there is an increasing interest in the development and improvement of methods that allow α-, β-, γ- and δ-tocopherol separation and hence their determination as single components.

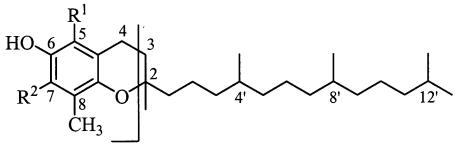
To obtain accurate determinations by MS/MS analyses trideuterated α-, β- and δ-tocopherol were needed as internal standards. In fact, on theoretical grounds, the molecular weight of the internal standard has to be increased, preferably by at least three mass units, to avoid the interference of natural isotopes of the analyte with the *m*/*z* value of the labeled compound.[8]

Several synthetic procedures for the preparation of polydeuterated tocopherols are known. The deuterium is usually introduced by the use of labeled formaldehyde and reducing reagents.[9] For the synthesis of trideuterated (all-*rac*)-α- and -β-tocopherol we used[10] a recently optimized procedure,[11] in which the corresponding ($^2H_2$)-morpholinomethyl tocopherol is prepared by a Mannich reaction and then reduced with $NaCNBD_3$.

The preparation of (all-*rac*)-δ-tocopherol containing labels in the aromatic methyl group required a totally different approach that we will describe in this paper as the first synthesis of (8-$^2H_3$)-(all-*rac*)-δ-tocopherol.

Results and Discussion

In order to avoid cross-talk phenomena and to follow the most abundant ion in quantitative analyses by tandem mass spectrometry, the labeling position was chosen on the basis of the tocopherol fragmentation pattern. The fragment resulting from loss of the aliphatic chain and breaking of the dihydropyran ring is the most abundant in this kind of analyses[12] (Figure 1).



Starting tocopherol	α	β	γ	δ
R^1	CH_3	CH_3	H	H
R^2	CH_3	H	CH_3	H
m/*z* of characteristic fragment	165	151	151	137

Figure 1. Fragments from tocopherols

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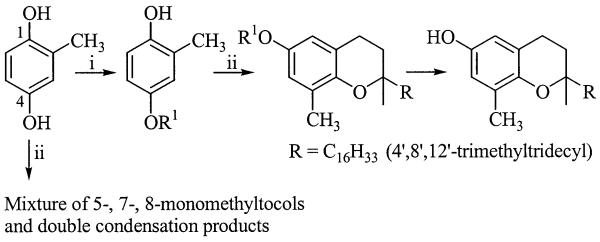
Hence we considered the introduction of convenient labels into the aromatic methyl group to be the most useful for our purposes.

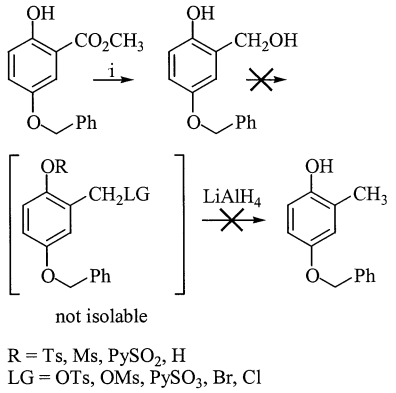
As, to the best of our knowledge, no method is known for the introduciton of a CH_3 (CD_3) group selectively in the 8-position of tocol, we envisaged the preparation of a suitable trideuterated aromatic building block and its subsequent acid-catalyzed condensation reaction with isophytol, in analogy to the preparation of (all-*rac*)-α-tocopherol.[13] It should be noted, however, that the adaptation of such methods to the synthesis of the lower homologue(s) is not trivial, and known procedures for the preparation of (all-*rac*)-δ-tocopherol on a laboratory scale are low-yielding.[14] Protection of the phenolic group in position 4 of the starting hydroquinone is generally required to avoid the formation of 5- and 7-monomethyl tocol regioisomers and possible double-alkylation products in the condensation step (Scheme 1).



Scheme 1. Key: i) selective protection; ii) acid (cat.), isophytol; iii) deprotection

Due to the small steric effect of the methyl group, selective protection of methylhydroquinone is not an easy task. TIPS and TBDMS have been employed as O-protective groups for this purpose. Good yields (70% using TBDMS chloride;[15] 79% with TIPS triflate/triethylamine/Et_2O/−75→20 °C) of the monoprotected derivatives were obtained starting from methylhydroquinone but the silyl groups were found to be removable under the acidic conditions used in the next step. Attempts to introduce the trityl group (Ph_3CCl/DMAP/Et_3N) gave low selectivity, which was even lower when the benzylic group (BnBr/K_2CO_3/acetone) was used.

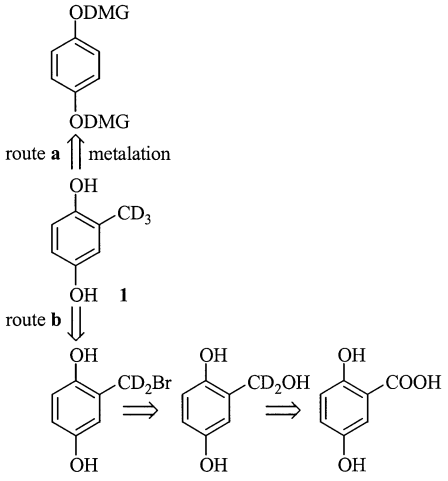
Considering those difficulties in direct selective monoprotection of methylhydroquinone, we looked for an alternative approach. Successful examples of the transformation of the least hindered phenolic group into a benzyl ether moiety in 2,5-dihydroxybenzoic acid or esters are known in the literature.[16] The *O*-benzyl group would represent a good protective group because of its stability during condensation reactions and the mild conditions that can be used for its subsequent removal. On a theoretical basis, the aromatic ester moiety can be reduced to a methyl group through a multi-step reduction sequence with the corresponding benzylic alcohol as an intermediate. Unfortunately, we were unable to synthesize the corresponding mesylate, tosylate, bromide, or chloride for the last step (Scheme 2, LG = leaving group). All such compounds proved to be very unstable. Neither isolation nor conducting the whole sequence including the reduction reaction in one pot were successful. Minami and Kijima have reported a simple reduction of 3,4,5-trimethoxysalicylic acid to 2,3,4-trimethoxyphenol using $NaBH_4$,[17] but preliminary experiments gave poor yields. These results prompted us to follow the monobenzoate route.[18]



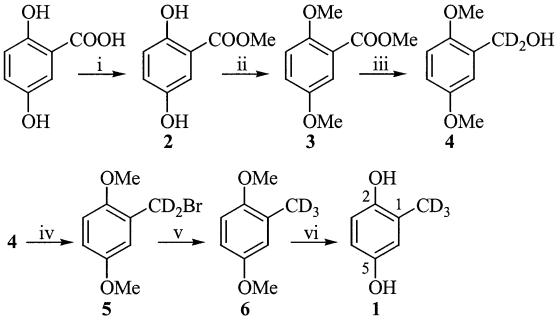
Scheme 2. Key: i) $LiAlH_4$, THF, 78%

The retrosynthetic scheme for the preparation of (α,α,α-$^2H_3$)-methylhydroquinone (**1**) by introduction of deuterium labels suggests two possible reaction pathways (Scheme 3): directed metalation[19] (route **a**) is a very powerful and easy synthetic technique, so it was the first to be investigated. Metalation reactions were performed using *N*,*N*-diisopropylcarbamoyl, methyl,[20] and methoxymethyl (MOM) as directing metalation groups (DMG) with the use of *n*BuLi as metalating agent and CH_3I as electrophile. Very good conversion was observed with the MOM and methyl groups (up to 92%; *n*BuLi 1.1 equiv., CH_3I 5 equiv., room temp.), but we were not able to separate the product from about 8−10% of the starting hydroquinone derivative. Therefore, for a clean synthesis of the desired **1**, we followed route **b**. Commercially available 2,5-dihydroxybenzoic acid was converted into the corresponding methyl ester **2** (Scheme 4), and the two phenolic groups in **2** were protected with methyl iodide to avoid unnecessary consumption of $LiAlD_4$ and to increase yields. Bis-ether **3** was then reduced to the benzylic alcohol **4** with $LiAlD_4$. Treatment of the latter with PBr_3 gave benzylic bromide[21] (**5**), which was reduced again with $LiAlD_4$ (→ **6**) and deprotected[22] with BBr_3, giving the pure trideuterated methylhydroquinone **1** in a 50% yield over six steps, with 98.5% isotopic purity (GC/MS).

It is noteworthy that protection of the phenolic OH groups as MOM ethers (which would be easier to cleave than methyl ether groups) was not suitable for the synthesis of the bromide from the benzylic alcohol. We also tried the conversion into the tosylate, mesylate, and chloride under various conditions, but we always obtained polymeric material. Considering the successful reaction performed on **5**, it is plausible to suppose an anchimeric assistance of the

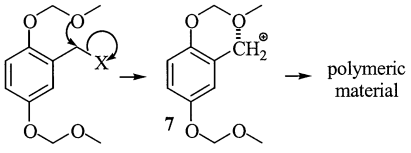


Scheme 3



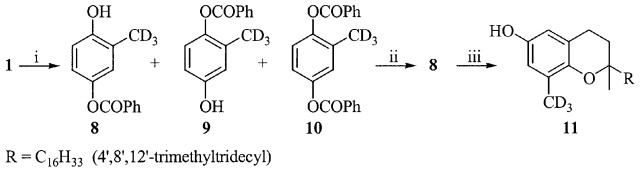
Scheme 4. Key: i) MeOH, H_2SO_4, 96%; ii) 1. NaH/THF 2. CH_3I, 92%; iii) $LiAlD_4$/THF, 92%; iv) PBr_3/CH_2Cl_2, sublimation, 82%; v) $LiAlD_4$/THF, 91%; vi) BBr_3/CH_2Cl_2, 82%

MOM group with the leaving group X, promoting the formation of a highly reactive carbocation **7** (Scheme 5) that gives rise to the polymeric material isolated.



Scheme 5

Methylhydroquinone (**1**) prepared according to Scheme 4 was then converted into a mixture of monoprotected benzoates **8** and **9** (ca. 65:35), and dibenzoate **10** (Scheme 6). For lab-scale isolation of monobenzoate **8**, the original procedure[18] was optimized (by using unlabeled material). After treatment with benzoyl chloride in pyridine, the 4-benzoyloxy derivative **8** was separated from **9** and **10** by fractional crystallization and careful flash chromatography in reasonable yield (45% on a 800-mg scale). Though this result is not exceptional, this procedure is the best up to now for the synthesis of 4-benzoyloxy-2-trideuteromethylphenol.



$R = C_{16}H_{33}$ (4',8',12'-trimethyltridecyl)

Scheme 6. Key: i) PhCOCl/Py, cryst.; ii) flash chromatography separation; iii) 1. $ZnCl_2$ (1.5 equiv.), *i*BuOAc, aq. HCl (cat.), isophytol, 2. KOH/MeOH, room temp.

For the following condensation step the conditions used for the preparation of (all-*rac*)-α-tocopherol[23] were applied. Although the $ZnCl_2$-catalyzed condensation of monobenzoate **8** with isophytol ran less smoothly, pure (8-$^2H_3$)-(all-*rac*)-δ-tocopherol (**11**) was obtained after saponification in 60% yield with a 98.5% deuteration of CD_3. No D_2 or D_1 analogs were detected by mass spectrometry analysis. It is assumed that the lower yield compared to the one obtained in α-tocopherol synthesis by the same procedure is due to partial ester hydrolysis and to the reduced reactivity of the mono-ester than the hydroquinone.

Conclusion

Unlike its α- and β-analogs, trideuterated δ-tocopherol labeled at the aromatic methyl group at the 8-position requires a complex multi-step synthesis. Optimization of each step was necessary to increase the overall yield. Hence different approaches were followed to obtain (α,α,α-$^2H_3$)-methylhydroquinone derivatives protected at the 4-position as key building blocks. Benzoylation gave the best results for selective protection, allowing the successful synthesis of pure (8-$^2H_3$)-(all-*rac*)-δ-tocopherol with a high percentage of deuteration (>98%). Introduction of a benzyl group at the 4-position is highly attractive too, but this proved to be difficult, as shown by the various procedures examined for the synthesis of the desired 4-benzyloxy-2-methylphenol. Further investigations following the Minami method, as well as other routes, are in progress.

The preparation of (8-$^2H_3$)-(all-*rac*)-δ-tocopherol is, to the best of our knowledge, the first synthesis ever reported concerning a deuterated δ-tocopherol. It allows accurate MS/MS quantitative determinations in different foodstuff matrices and can be used as a tool to develop metabolic studies of the non-vitaminic activity of δ-tocopherol.

Experimental Section

General: All reactions were performed under an inert atmosphere (argon or N_2). Melting points were measured on a Reichert-Jung Thermovar apparatus. ^1H and ^{13}C NMR spectra were recorded on a Varian Gemini 200 spectrometer (200 MHz and 50.3 MHz, respectively), in $CDCl_3$ solution with Me_4Si or $CHCl_3$ as internal standards. ^2H NMR spectra were recorded on a Varian VXR 300 spectrometer (46 MHz for ^2H) in $CHCl_3$ with $CDCl_3$ as internal

standard. Chemical shifts are expressed on the δ scale (ppm). GLC analyses were performed on a Perkin−Elmer 8600 Model instrument, equipped with a DB1 column (30 m × 0.52 mm, 5 μm film thickness), and on a Perkin−Elmer AutoSystem XL Model instrument with a BP-1 capillary column (12 m × 0.25 mm, 0.25 μm film thickness), both with He as carrier gas and with a flame ionization detector. The atmospheric pressure chemical ionization mass spectra were acquired on a Perkin−Elmer Sciex API III Plus mass spectrometer. GC/MS analyses were recorded on a Saturn 2000 GC-MS/MS Varian Chromatography System mass spectrometer connected to a 3800 Varian gas chromatograph equipped with a DB-5 capillary column (30 m × 0.25 mm, 0.25 μm film thickness). Commercial reagents and solvents were purchased from Aldrich, Fluka, or Merck, and purified by standard methods when necessary. Tetrahydrofuran was distilled from over Na/K alloy before use. Dichloromethane was distilled from over P_2O_5. Pyridine was distilled from over potassium hydroxide and stored over KOH. LiAlD_4 (96 atom% D) and CD_3I (≥ 99.5 atom% D) were acquired from Aldrich. Deuterated paraformaldehyde $(CD_2O)_n$ (≥ 99.5 atom% D) was purchased from C/D/N Isotopes (Canada). All other commercial reagents were used without further purification. Column chromatography was performed on silica gel 60 (70−230 mesh and 230−400 mesh). TLC was performed on silica gel Macherey−Nagel Alugram Sil G/UV_{254} (0.20 mm). Hex means *n*-hexane. Usual workup involved three extractions into the solvent specified. The organic extracts were combined, washed with water until the aqueous phase was neutral, dried over Na_2SO_4, filtered and then concentrated on a rotary evaporator under vacuum. The residue was further dried to constant weight under high vacuum. All yields given refer to isolated yields.

Methyl 2,5-Dihydroxybenzoate (2): 2,5-Dihydroxybenzoic acid (6.73 g, 43.70 mmol) was dissolved in MeOH (45 mL, HPLC grade, 1.11 mol, 25 equiv.) and mixed with 95% H_2SO_4 (3 mL, 53.22 mmol, 1.2 equiv.). The solution was refluxed overnight,[24] after which time no starting acid could be detected by TLC (Hex/EtOAc, 4:1). The solvent was then evaporated and water added. Usual workup in diethyl ether gave pure **2** (7.05 g, 96% yield) as a white solid. M.p. 84−86 °C; NMR ($CDCl_3$/TMS): δ = 3.91 (s, 3 H, 1-CH_3), 5.49 (s, 1 H, 5-OH), 6.87 (d, *J* = 8.6 Hz, 1 H, H-3), 7.02 (dd, J_1 = 3.0 Hz, J_2 = 8.5 Hz, 1 H, H-4), 7.28 (d, *J* = 3.6 Hz, 1 H, H-6), 10.39 (s, 1 H, 2-OH) ppm.

Methyl 2,5-Dimethoxybenzoate (3): A magnetically stirred suspension of dry NaH (1.84 g, 75 mmol) in dry THF (15 mL) was cooled to 0 °C. A solution of ester **2** (4.2 g, 25 mmol) in dry THF (15 mL) was added dropwise over 20 min. The reaction mixture was allowed to warm to room temp. and stirred for an additional hour. The mixture was cooled again to 0 °C, and a solution of CH_3I (6.23 mL, 100 mmol) in dry THF (15 mL) was added dropwise. The reaction mixture was allowed to warm to room temp. and stirred overnight. The end of the reaction was confirmed by TLC (Hex/EtOAc, 5:1). Water (50 mL) was added, and the THF evaporated. After usual workup in diethyl ether and column chromatography (Hex/EtOAc, 4:1) pure **3** (4.55 g, 92% yield) was obtained as a yellowish oil. ^1H NMR ($CDCl_3$/TMS): δ = 3.65 (s, 3 H, 1-CH_3), 3.82 (s, 3 H, 5-CH_3), 3.87 (s, 3 H, 2-CH_3) 6.87 (d, *J* = 8.6 Hz, 1 H, H-3), 7.01 (dd, J_1 = 3.0 Hz, J_2 = 8.5 Hz, 1 H, H-4), 7.32 (d, *J* = 3.6 Hz, 1 H, H-6) ppm.

(α,α-$^2H_2$)-2,5-Dimethoxybenzyl Alcohol (4): A magnetically stirred suspension of LiAlD_4 (1.0 g, 23.82 mmol) in dry THF (15 mL) was cooled to 0 °C. A solution of ester **3** (3.95 g, 20.15 mmol) in dry THF (15 mL) was added dropwise over 20 min, and the reaction mixture was stirred overnight. The end of the reaction was confirmed by TLC (Hex/EtOAc, 5:1). After cooling to 0 °C and dropwise addition of water (50 mL), the reaction mixture was stirred for an additional 30 min and then filtered. The white aluminate salts were washed with diethyl ether, and the organic solvent was evaporated. Usual workup in diethyl ether of the resulting aqueous phase gave pure **4** (3.15 g, 92% yield) as a yellowish oil. ^1H NMR ($CDCl_3$/TMS): δ = 2.74 (br. s, 1 H, 1-OH) 3.72 (s, 3 H, 5-CH_3), 3.80 (s, 3 H, 2-CH_3), 6.55−6.95 (m, 3 H, H_{arom}) ppm; no detectable benzyl proton signal at δ = 4.64 ppm. ^2H NMR ($CHCl_3$): δ = 130.0 (C-1), 151.4 (C-2), 114.4 (C-3), 112.7 (C-4), 153.6 (C-5), 110.9 (C-6), 55.6 (5-CH_3), 55.7 (2-CH_3), 60.1 (m, CD_2OH) ppm.

(α,α-$^2H_2$)-2,5-Dimethoxybenzyl Bromide (5): A magnetically stirred solution of **4** (2.77 g, 16.29 mmol) in dry CH_2Cl_2 (25 mL) was cooled to 0 °C, and a solution of PBr_3 (610 μL, 6.49 mmol) in dry CH_2Cl_2 (15 mL) was added dropwise over 20 min. The reaction mixture was allowed to warm to room temp. and stirred overnight, after which time the end of the reaction was confirmed by TLC (Hex/EtOAc, 3:1). After usual workup in CH_2Cl_2 the crude product was purified by sublimation (90−100 °C, 0.1 Torr), giving pure **5** (3.11 g, 82% yield) as a white solid. M.p. 74−76 °C. ^1H NMR ($CDCl_3$/TMS): δ = 3.76 (s, 3 H, 5-CH_3), 3.86 (s, 3 H, 2-CH_3), 6.70−6.93 (m, 3 H, aromatic H) ppm; no detectable benzyl proton signal at δ = 4.55 ppm. ^2H NMR ($CHCl_3$): δ = 4.48 (s, benzylic D) ppm. ^{13}C NMR ($CDCl_3$): δ = 26.8 (C-1), 153.4 (C-2), 114.9 (C-3), 112.0 (C-4), 151.7 (C-5), 116.3 (C-6), 55.7 (5-CH_3), 56.2 (2-CH_3), 29.2 (m, CD_2Br) ppm.

(α,α,α-$^2H_3$)-1,4-Dimethoxy-2-methylbenzene (6): A magnetically stirred suspension of LiAlD_4 (630 mg, 15 mmol) in dry THF (15 mL) was cooled to 0 °C, and a solution of bromide **5** (3.07 g, 13.17 mmol) in dry THF (20 mL) was added dropwise. The reaction mixture was allowed to warm to room temp. and stirred overnight; the end of the reaction was confirmed by TLC (Hex/EtOAc, 5:1). After cooling to 0 °C and dropwise addition of water (50 mL), the reaction mixture was filtered by suction filtration. The white aluminate salts were washed with diethyl ether, and the organic solvent was evaporated. Usual workup in diethyl ether of the resulting aqueous phase and bulb-to-bulb distillation (50−60 °C, 1 Torr) gave pure **6** (1.86 g, 91% yield) as a yellowish oil. ^1H NMR ($CDCl_3$/TMS): δ = 3.76 (s, 3 H, 5-CH_3), 3.80 (s, 3 H, 2-CH_3) 6.65−6.85 (m, 3 H, aromatic H) ppm; no detectable aromatic methyl proton signal at δ = 2.24 ppm. ^2H NMR ($CHCl_3$): δ = 2.23 (s, CD_3) ppm.

(α,α,α-$^2H_3$)-Methylhydroquinone (1): A magnetically stirred solution of **6** (1.3 g, 8.39 mmol) in dry CH_2Cl_2 (4 mL) was cooled to −78 °C, and BBr_3 (27.7 mL of a 1 M dichloromethane solution, 27.7 mmol) was added dropwise. The reaction mixture was allowed to warm to room temp. and stirred for 15 h; TLC (Hex/EtOAc, 3:1) confirmed the end of the reaction. Next sat. aq. NaCl solution was carefully added to the mixture. Usual workup in diethyl ether and column chromatography (CH_2Cl_2/EtOAc, 6:1) afforded **1** (870 mg, 82% yield) as a white solid. M.p. 126−127.5 °C. 98.5% deuteration of CD_3 (MS). ^1H NMR ($CDCl_3$/TMS, 45 °C): δ = 4.34 (s, 2 H, 2 OH), 6.55 (dd, J_1 = 3.5 Hz, J_2 = 2.1 Hz, 1 H, H-4), 6.62 (d, *J* = 2.1 Hz, 1 H, H-3), 7.28 (d, *J* = 3.6 Hz, 1 H, H-6) ppm; no detectable aromatic methyl proton signal at δ = 2.12 ppm. ^2H NMR ($CHCl_3$): δ = 2.11 (s, CD_3) ppm. GC/MS (DB-5, 30 m, 70→300 °C, 20 °C/min, 10 min a 300 °C): t_R(**1**) = 3.9 min. ESI-MS: *m*/*z* = 127 (100), 98 (35).

(α,α,α-$^2H_3$)-4-Benzoyloxy-2-methylphenol (8): A solution of benzoyl chloride (0.76 mL, 6.49 mmol, 1.03 mol equiv.) in dry pyridine (15 mL) was added dropwise at −10 °C to a stirred solution of

hydroquinone **1** (800 mg, 6.3 mmol) in dry pyridine (15 mL). The reaction mixture was stirred for 3 h at −10 °C, allowed to warm to room temp., and then acidified with 3 N HCl. After usual workup in diethyl ether, the crude product was dissolved in MeOH (5 mL) and kept at −20 °C overnight. The crystals formed (dibenzoate derivative **10**) were filtered and washed with cold MeOH (5−7 mL). The filtrate and combined washings were evaporated, and the residue (GC, **8**:**9** 65%:35%) was purified by careful flash chromatography (90 g SiO_2, Hex/EtOAc, 4:1). From the first eluting zone 370 mg of **8** (GC, 92%) was obtained, and this was recrystallized from 4 mL of Hex/EtOAc (4:1), giving 330 mg of **8** (purity 98.2%, GC). From the second eluting zone 500 mg (GC, **8**:**9** 70:30) was obtained that gave 320 mg of **8** (purity 99%, GC) after a second flash chromatography. Overall yield 45%. M.p. 112−114 °C. ^{1}H NMR ($CDCl_3$/TMS): 5.15 (br. s, OH, 1 H), 6.7 (d, J = 8.6 Hz, 1 H), 6.86 (dd, J_1 = 2.6, J_2 = 8.6 Hz, 1 H), 6.96 (d, J = 2.6 Hz, 1 H), 7.47−7.7 (m, 3 H), 8.19 (m, 2 H) ppm; no detectable aromatic methyl proton signal at δ = 2.23 ppm. ^{2}H NMR (46 MHz, $CHCl_3$): δ = 2.19 (s) ppm. ^{13}C NMR ($CDCl_3$): δ = 15.9 (m, CD_3), 115.9, 120.2, 124.5, 125.8, 128.9, 129.8, 130.6, 134.1, 144.6, 152.2, 166.2 ppm. GC on BP-1, 12 m, 70 →300 °C, 20 °C/min, 10 min at 300 °C: t_R(**8**) = 8.15 min, t_R(**9**) = 7.95 min. APCI-MS (in MeOH): m/z = 232 [M + H$^+$].

(8-$^2H_3$)-(all-*rac*)-δ-Tocopherol (11): A solution of isophytol (0.57 mL, 1.62 mmol) in *i*BuOAc (5 mL) was added dropwise very slowly (over 3 h) at 50 °C to a stirred mixture of monobenzoate **8** (250 mg, 1.08 mmol), 0.5 mL of aq. HCl >36.5% and anhydrous $ZnCl_2$ (225 mg, 1.62 mmol) in *i*BuOAc (5 mL). The reaction mixture was stirred for a further 3 h at 50 °C and then allowed to cool to room temp. After workup by extraction with toluene/water, the resulting crude brownish mixture was saponified by stirring for 4.5 h at room temp. with methanolic 1 M KOH (12 mL). The mixture was then acidified with 2 N HCl, and the MeOH was evaporated. Usual workup and flash chromatography (Hex/EtOAc, 9:1) gave pure **11** (260 mg, 60% yield) as a brown-yellow oil. 98.5% deuteration of CD_3 (MS). ^{1}H NMR ($CDCl_3$/TMS): δ = 0.7−1.9 (m, H_2-C(3), H_3-C(2a) and $C_{16}H_{33}$ chain), 2.65 (t, J = 7 Hz, H_2-C(4), 2 H), 4.4 (br. s, OH, 1 H), 6.38 [d, J = 2.6 Hz, H-C(5), 1 H], 6.47 [s, 1 H, H-C(7)] ppm; no detectable aromatic 8-methyl proton signal at δ = 2.09 ppm. ^{2}H NMR (46 MHz, $CHCl_3$): δ = 2.08 [s, D_3C-C(8)] ppm. ^{13}C NMR ($CDCl_3$): δ = 147.8, 146, 127.1, 121.3, 115.8, 112.5, 75.5, 40.1, 39.4, 37.3−37.6, 32.8, 32.7, 31.4, 28, 24.8, 24.4, 24, 22.7, 22.6, 22.5, 21, 19.5−19.7 ppm. APCI-MS (in MeOH): m/z = 406 [M + H$^+$], 140 (fragmentation see Figure 1). $C_{27}H_{43}D_3O_2$ (405.2): calcd. C 79.94, H 12.17; found C 79.91, H 12.19.

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