Approaches to the Preparation of 4-Benzyloxy-2-(α,α,α-D_3)methylphenol, a Building Block for Labeled δ-Tocopherol, and a New Synthesis of *R*,*R*,*R*-5-D_3-α-Tocopherol

Francesco Mazzini,[a] Alessandro Mandoli,[a] Piero Salvadori,*[a] Thomas Netscher,[b] and Thomas Rosenau[c]

Keywords: Natural products / Tocopherols / Deuterium labeling / Chroman ring / Reduction

Different routes are described for the synthesis of 4-benzyloxy-2-D_3-phenol, a key building block in the preparation of D_3-δ-tocopherol. Conditions for the improvement of Minami's reduction are also given, allowing a straightforward route to the title compound and a new synthesis of *R*,*R*,*R*-5-D_3-α-tocopherol in good yields, starting from widely available *R*,*R*,*R*-α-tocopherol.

Introduction

Vitamin E is the most important fat-soluble, chain-breaking antioxidant. The term vitamin E covers all tocol and tocotrienol derivatives that exhibiting the biological activity of α-tocopherol.[1] There is growing interest in a deep and thorough study of α-tocopherol lower homologues,[2] γ- and δ-tocopherol. Despite their much lower plasma concentration and bioactivity compared to α-tocopherol, it is important to have a full understanding of their unique properties that are not shared by α-tocopherol, and which may be very important to human health. The strong interest in these biologically relevant compounds prompted the development of a synthesis of their labeled analogues to greatly help in in vivo studies on the metabolic mechanism and allow very accurate evaluation of their content in biological and food matrices. The utilization of stable isotope-labeled analogues greatly facilitates the carrying out of such studies.[3] In fact, it is a powerful tool in terms of both specificity and sensitivity, acting as a probe in the body and as an internal standard for accurate quantitative determination by specific techniques such as mass spectrometry,[4−6] which are being used more and more for the characterization of such complex matrices.

As the building of the chroman and aliphatic side-chain systems has already been reported in various and efficient ways,[7] the key point in planning a trideuteromethyl δ-tocopherol synthesis in either a racemic or a stereoisomerically pure form can be envisaged in the preparation of a D_3-hydroquinone derivative properly protected on the oxygen in the 4-position.

Here we describe a very easy and high yielding procedure for the synthesis of 2-D_3-4-benzyloxyphenol (**1**) that dramatically improves our previous preparation of 8-D_3-δ-tocopherol.[8] Moreover, we report the application of the same simple route for an alternative synthesis of 5-D_3-α-tocopherol.

Results and Discussion

In reporting the first synthesis of D_3-δ-tocopherol,[8] we described the difficulties encountered in attempting the preparation of the labeled methylhydroquinone derivative **1**. Protection of the phenolic group in the 4-position of the hydroquinone ring is required to avoid the formation of 5- and 7-monomethyl regioisomers and possible double-alkylation products[9] in the subsequent condensation step with isophytol for the construction of the chroman ring (Figure 1). Benzyl would be a good protective group for this purpose because of its stability in the condensation reaction[9] and the mild conditions used for its subsequent removal, but our previous attempts to perform the reduction of methyl 5-benzyloxy-2-hydroxy benzoate to **1**, via the cor-

[a] Dipartimento di Chimica e Chimica Industriale, Università di Pisa,
Via Risorgimento 35, 56126 Pisa, Italy
Fax: (internat.) + 39-050-2219-918-260
E-mail: psalva@dcci.unipi.it
[b] Research and Development, DSM Nutritional Products,
P.O. Box 3255, 4002 Basel, Switzerland
Fax: (internat.) + 41-061-687-2201
E-mail: thomas.netscher@dsm.com
[c] Institute of Chemistry, University of Agricultural Sciences,
Muthgasse 18, 1190 Vienna, Austria
Fax: (internat.) + 43-1-36006-6059
E-mail: thomas.rosenau@boku.ac.at

  DOI: 10.1002/ejoc.200400535

responding benzylic alcohol as an intermediate, gave very poor results.[8]

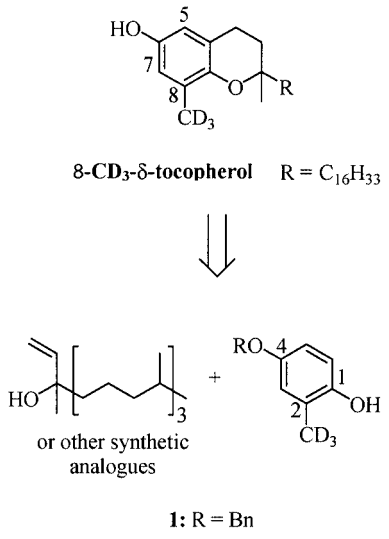
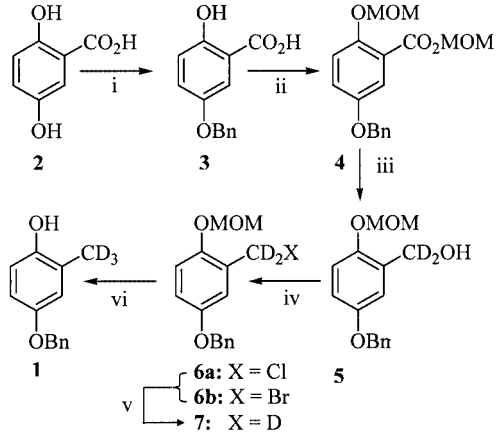


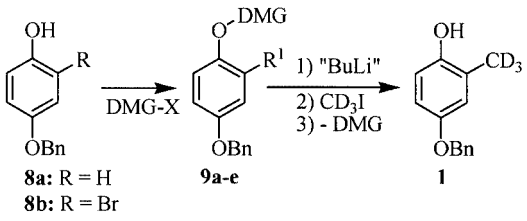
Figure 1. Retrosynthesis of ring-labeled δ-tocopherol

In reinvestigating this route, we became interested in a paper by Versteeg et al. where the trapping of methanesulfonates analogous to **5** as the corresponding bromide was briefly mentioned.[10] Therefore, **5** was prepared from the acid **2** and reacted with $MeSO_2Cl$, 2,6-lutidine and excess LiBr to afford a mixture of chlorinated and brominated products **6a** and **6b**, respectively. This mixture proved to be unstable on standing, but its immediate reduction by $LiAlD_4$ provided, after deprotection, the desired hydroquinone **1** in 72% overall yield from **5** (Scheme 1).



Scheme 1. i) NaH, DMF then BnBr, 86%; ii) NaH, DMF then MOMCl, quant.; iii) $LiAlD_4$, THF, 86%; iv) $MeSO_2Cl$, 2,6-lutidine, excess LiBr, THF, 85%; v) $LiAlD_4$, THF, 90%; vi) MeOH, cat. *p*-TsOH, 50 °C, 3 h, 95%

Although most of the steps of this route proceed in high yields, we also considered the use of a directed metallation strategy[11] in order to shorten the synthesis of **1**. For this purpose, *N*,*N*-diisopropylcarbamoyl, *N*,*N*-diethylcarbamoyl, methyl and methoxymethyl (MOM) derivatives (**9a**−**d**) of the commercially available hydroquinone monobenzyl ether **8** were prepared and subjected to several alkylation runs, using *n*BuLi or *t*BuLi as the metallating agent and CD_3I as the electrophile. In the best case 70% conversion of **9d** into the trideuteromethyl derivative was achieved, but, in general, the separation of the desired product from the unchanged starting material was not possible. However, we succeeded in preparing pure **1** by using a bromine−lithium exchange at low temperature on the brominated derivative **9e**.[12] Addition of a slight excess (1.4 equivalents) of *n*BuLi followed by the prompt addition of six equivalents of CD_3I gave compound **1** in 79% overall yield from **8b**, after removal of the MOM group and purification by column chromatography (Scheme 2).

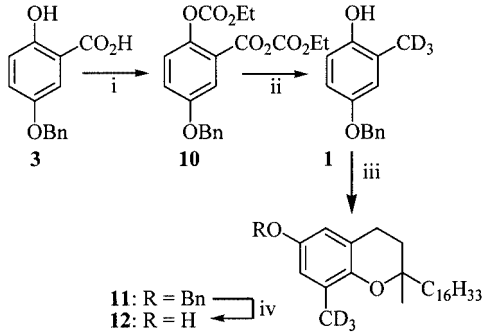


9a: R^1 = H, DMG = Et_2NCO
9b: R^1 = H, DMG = iPr_2NCO
9c: R^1 = H, DMG = CH_3
9d: R^1 = H, DMG = CH_3OCH_2
9e: R^1 = Br, DMG = CH_3OCH_2

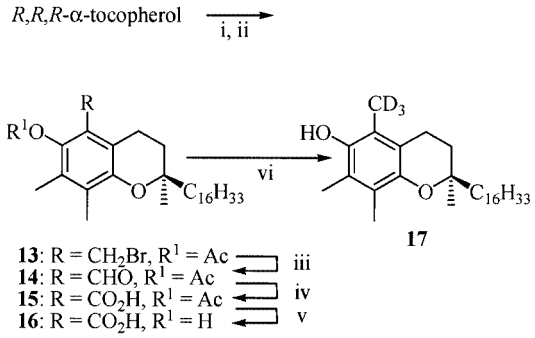
Scheme 2. Preparation of **1** by directed metallation (DMG = directing metallation group)

Although the two routes just described were already satisfactory, we eventually found a much more effective route to **1** based on the report by Minami and Kijima of the reduction of salicylic acids to the corresponding 2-methylphenols.[13] According to this method, the salicylic acid is converted upon reaction with ethyl chloroformate into a bis-ethoxycarbonyl derivative, whose treatment with sodium borohydride in aqueous THF directly affords the 2-methylphenol in moderate to good yields. The latter step is supposed to proceed through an initial reduction of the carboxylic site of the mixed anhydride moiety to the corresponding benzyloxy anion, followed by migration of the residual carbonate group from the phenolic to the alcoholic oxygen atom. Subsequent cleavage of the benzylic C−O bond of the resulting phenoxide derivative would then provide a highly reactive *o*-quinone methide, whose reduction by the borohydride eventually leads to the methylphenol product.[14]

Preliminary experiments on **3** carried out according to this protocol (Scheme 3) revealed that, while the formation of the intermediate bis-carbonate **10** was quantitative, only a 50% yield of the desired methylphenol was obtained under Minami's original reduction conditions (4 equiv. $NaBH_4$, H_2O/THF 1:1 as solvent). At first, we supposed that hydrolysis of the mixed anhydride **10** back to the acid could take place in the basic aqueous medium. Therefore,



Scheme 3. i) $ClCO_2Et$, Et_3N, 0 °C, 2 h; ii) $NaBD_4$, 8 equiv., D_2O/THF, 0 °C, 3 h, 89%; iii) $ZnCl_2$, 1.5 equiv., *i*BuOAc, aq. HCl_{cat}, isophytol, 72%; iv) 5% Pd/C, H_2, EtOAc, overnight, quant.



Scheme 4. i) Br_2, hexane, room temp., 3 h; ii) Ac_2O, AcOH, CH_2Cl_2, H_2SO_4 cat., room temp., overnight, 82% over 2 steps; iii) NMO, 4 equiv., CH_3CN, room temp., overnight, 92%; iv) NH_2SO_3H, $NaClO_2$, 1,4-dioxane/H_2O, room temp., 50 min, 96%; v) KOH 2 M, MeOH, 50 °C, 2 h, 93%; vi) a. $ClCO_2Et$, Et_3N, 0 °C, 2 h, quant. b. $NaBD_4$, 8 equiv., D_2O/THF, 0 °C, 3 h, 72%

we tried changing the solvent to a mixture of THF and MeOH, but under these conditions only methyl ester and ethyl carbonate derivatives were obtained. Thus, water seems to play an essential role in the reaction, as noted before by Mitchell.[14] In a further experiment, the dropwise addition of water to a suspension of THF and $NaBH_4$ afforded the methylphenol **1** in 35% yield, together with the corresponding benzylic alcohol in 45% yield. While this was still unsatisfactory from a synthetic point of view, the formation of the latter product nonetheless offered a clue to the low to moderate yields reported in Minami's paper for most of the substrates. A possible interpretation is that the postulated *o*-quinone methide intermediate undergoes attack by water instead of reduction by the borohydride. Hence, in order to steer the reaction towards the formation of the methylphenol product, we investigated the effect of increasing the amount of reducing agent, by carrying out several experiments in THF/H_2O (1:1), with between five and ten equivalents of $NaBH_4$. Under these conditions the yields of product increased starting from six equivalents, reaching a maximum of 89% with eight equivalents. No further improvements were observed with larger amounts of $NaBH_4$. Having optimized the procedure, the reduction step of **10** was repeated with $NaBD_4$ in THF/H_2O or, more effectively, in THF/D_2O to prevent H/D scrambling. With this route, **1** could be prepared with an excellent 98% isotope purity (GC-MS) and 77% overall yield from **2**, through three easy and highly efficient steps.

After acid-catalyzed condensation of **1** with isophytol, mild Pd/C debenzylation smoothly provided *rac*-D_3-δ-tocopherol **12**, without any loss of the level of deuteration (Scheme 3).

Finally, the excellent results achieved in the modified Minami reduction prompted us to apply the same procedure to the easily accessible γ-tocopherol-5-carboxylic acid **16**.[15] Following a known sequence, α-tocopherol of natural origin was converted into **16**, by benzylic bromination and a two-step oxidation,[16–18] and **16** was then reduced with $NaBD_4$ to the enantiomerically pure *R*,*R*,*R*-5-D_3-α-tocopherol **17** in 72% yield from **16** (Scheme 4).

Conclusions

Different approaches have been explored for the preparation of labeled 4-benzyloxy-2-methylphenol (**1**), which is a key building block in the synthesis of deuterated δ-tocopherol. In particular, after optimization of Minami's original procedure for the reduction of salicylic acids to 2-methylphenols, a very simple high yielding and scalable route was set up, allowing the preparation of **1** in three steps from commercially available hydroxysalicylic acid (**2**). Consequently, the synthesis of labeled *rac*-δ-tocopherol was dramatically improved compared with our previously reported route.[8] The modified Minami protocol also proved effective with other salicylic acid systems, providing a valid alternative for the preparation of *R*,*R*,*R*-5-D_3-α-tocopherol, starting from widely available α-tocopherol.

Experimental Section

All reactions were performed under an inert atmosphere (Ar or N_2). ^1H and ^{13}C NMR spectra were recorded on a Varian Gemini 200 spectrometer (200 MHz and 50.3 MHz, respectively), in $CDCl_3$ or CD_3OD 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). The APCI 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). Optical purity was checked by chiral HPLC analyses performed using a Jasco PU-980 system equipped with a Jasco 821-FP fluorimetric detector (λ_{ex} = 298 nm, λ_{em} = 345 nm) on a Chiralcel OD-H column (hexane/2-propanol, 200:1). Commercial reagents and solvents were purchased from Aldrich, Fluka, or Merck, and purified by standard methods when necessary. THF was distilled from Na/K alloy before use. Hexane was distilled from Na. $LiAlD_4$ (96 atom-% D), $NaBD_4$ (98 atom-% D), D_2O (99.8 atom-% D)and CD_3I (≥ 99.5 atom-% D) were purchased from Aldrich. 2*R*,4′*R*,8′*R*-α-Tocopherol (Covitol F1490) was purchased from

Henkel. 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). All yields given refer to isolated yields.

5-Benzyloxy-2-hydroxybenzoic Acid (3): A solution of **2** (9.24 g, 60 mmol) in DMF was added dropwise (30 mL) to a suspension of dry NaH (3.35 g, 132 mmol, 2.2 equiv.) in DMF (50 mL). After stirring for 2 h at room temperature, a solution of benzyl bromide (7.13 mL, 60 mmol, 1 equiv.) in DMF (15 mL) was added dropwise. After 2 h TLC (Hex/EtOAc/AcOH, 3:1:0.005) confirmed complete conversion of the starting acid. Water was added, the reaction mixture acidified to pH 3 with 1 M HCl and extracted with Et_2O (3 × 100 mL). The combined organic extracts were subsequently washed to neutrality with water, dried over Na_2SO_4 and finally concentrated under reduced pressure. Recrystallization of the crude product from $CHCl_3$ (60 mL) afforded 6.45 g of **3** as a white solid. A further 3.71 g were obtained from a second recrystallization from $CHCl_3$ (15 mL). The residue was purified by column chromatography (Hex/EtOAc/AcOH, 4:1:0.005) to give a further 1.7 g. Overall yield of **3**: 86%. ^{1}H NMR ($CDCl_3$/TMS): δ = 5.06 (s, 2 H, $PhCH_2$), 6.95 (d, J = 9.2 Hz, 1 H, ArH), 7.21 (dd, J_1 = 3.2, J_2 = 9.0 Hz,1 H, ArH) 7.3−7.45 (m, 6 H, ArH), 9.98 (s, 1 H, OH) ppm. ^{13}C NMR ($CDCl_3$): δ = 70.9, 110.7, 113.8, 118.9, 126.5,127.6, 128.1, 128.6, 136.6, 151.3, 157, 174.2 ppm.

Methoxymethyl 5-Benzyloxy-2-methoxymethoxybenzoate (4): A solution of **3** (4.88 g, 20 mmol) in DMF (30 mL) was added dropwise to a suspension of dry NaH (1.26 g, 50 mmol, 2.5 equiv.) in DMF (20 mL). After stirring for 1 h at room temperature, a solution of MOMCl (3.8 mL, 50 mmol, 2.5 equiv.) in DMF (15 mL) was added dropwise, and the resulting mixture was stirred overnight. Complete conversion of the starting phenol was confirmed by TLC (Hex/EtOAc, 7:1). Water was added (50 mL) and the reaction mixture extracted with Et_2O (3 × 100 mL). The combined organic extracts were subsequently washed to neutrality with water and dried over Na_2SO_4. Evaporation to dryness gave **4** (6.6 g, ≈ 100%) as yellow oil. ^{1}H NMR ($CDCl_3$/TMS): δ = 3.51 (s, 3 H, H_3COCH_2OAr), 3.53 (s, 3 H, H_3COCH_2OCO), 5.03 (s, 2 H, $PhCH_2$), 5.17 (s, 2 H, CH_3OCH_2OAr), 5.44 (s, 2 H, CH_3OCH_2-OCO), 7.1−7.6 (m, 8 H, ArH) ppm.

5-Benzyloxy-2-methoxymethoxy-(α,α-D_2)benzyl Alcohol (5): A solution of **4** (6.56 g) in dry THF (20 mL) was added dropwise at 0 °C to a stirred suspension of $LiAlD_4$ (1.4 g, 36.8 mmol) in dry THF (15 mL). The mixture was then warmed to room temp. and stirred for 3 h. TLC (Hex/EtOAc, 3:1) showed the complete conversion of reagent. The mixture was neutralized with water and NH_4Cl, and the THF evaporated off. The aqueous layer was filtered and extracted with Et_2O (3 × 100 mL), and the combined organic extracts were subsequently washed to neutrality with water and dried over Na_2SO_4. The solution was concentrated in vacuo to give **5** (4.72 g, 86% yield) as a pale-yellow solid. ^{1}H NMR ($CDCl_3$/TMS): δ = 2.8 (br. s, 1 H, OH), 3.49 (s, 3 H, H_3COCH_2OAr), 5.03 (s, 2 H, $PhCH_2$), 5.12 (s, 2 H, CH_3OC-H_2OAr), 6.83 (dd, J_1 = 3.2, J_2 = 9.2 Hz, 1 H, ArH), 6.9−7.06 (m, 2 H, ArH), 7.3−7.46 (m, 5 H, ArH) ppm. ^{2}H NMR ($CHCl_3$): δ = 4.63 (s, benzylic D) ppm. ^{13}C NMR ($CDCl_3$): δ = 56.0, 61.2 (m), 70.3, 95.4, 114.3, 115.2, 115.8, 127.3, 127.7, 128.4, 131.5, 137, 149, 153.8 ppm.

5-Benzyloxy-2-methoxymethoxy-(α,α-D_2)benzyl Bromide/Chloride Mixture (6a/6b): LiBr (13 g, 150 mmol, 10 equiv.), **5** (4.11 g, 15 mmol), 2,6-lutidine (3.48 mL, 30 mmol, 2 equiv.), MsCl (2.34 mL, 30 mmol, 2 equiv.) and dry THF (60 mL) were stirred at room temp. for 4 h. Water was then added and the THF evaporated off. The mixture was extracted with Et_2O (3 × 100 mL), the combined organic extracts were subsequently washed to neutrality with water, dried over Na_2SO_4 and concentrated to dryness. Purification by column chromatography (Hex/EtOAc, 4:1) afforded a mixture of **6a** and **6b** (4.32 g; Br/Cl = 77:23 by NMR) in 85% yield with respect to **5**. ^{1}H NMR ($CDCl_3$/TMS): δ = 3.5 and 3.51 (s, H_3CO-CH_2OArX), 5.01 and 5.02 (s, $PhCH_2$), 5.18 and 5.2 (s, CH_3O-CH_2OAr), 6.85−7.5 (m, ArH) ppm. ^{2}H NMR ($CHCl_3$): δ = 4.51 (s, $ArCD_2Br$), 4.63 (s, $ArCD_2Cl$) ppm.

4-Benzyloxy-1-methoxymethoxy-2-(D_3)methylbenzene (7) from 6a/6b: A solution of **6a/6b** (3.22 g) in dry THF (15 mL) was added dropwise to a stirred suspension of $LiAlD_4$ (940 mg, 24.6 mmol, 2.5 equiv.) in dry THF (20 mL). After 4 h, TLC (Hex/EtOAc, 20:1) confirmed the end of the reaction. The mixture was neutralized with water and NH_4Cl, and the THF evaporated off. The aqueous layer was filtered and extracted with Et_2O (3 × 100 mL), and the combined organic extracts were subsequently washed to neutrality with water and dried over Na_2SO_4. Evaporating to dryness afforded **7** (2.25 g, 90% yield) as a yellow oil. ^{1}H NMR ($CDCl_3$/TMS): δ = 3.56 (s, 3 H, H_3COCH_2OAr), 5.06 (s, 2 H, $PhCH_2$), 5.19 (s, 2 H, CH_3OCH_2OAr), 6.82 (dd, J_1 = 3.1, J_2 = 8.8 Hz, 1 H, ArH), 6.91 (d, J = 3.2 Hz, 1 H, ArH), 7.05 (d, J = 8.8 Hz, 1 H, ArH), 7.35−7.55 (m, 5 H, ArH) ppm. ^{2}H NMR ($CHCl_3$): δ = 2.30 (s, $ArCD_3$) ppm.

4-Benzyloxy-2-bromo-1-methoxymethoxybenzene (9e): Compound **8b** was prepared following the procedure reported by Dodsworth et al.[19] Its MOM derivative **9e** was obtained as described for the preparation of **4** in 98% yield. ^{1}H NMR ($CDCl_3$/TMS): δ = 5.00 (s, 2 H, $PhCH_2$), 3.51 (s, 3 H, H_3COCH_2OAr), 4.99 (s, 2 H, $PhCH_2$), 5.15 (s, 2 H, CH_3OCH_2OAr), 6.85 (dd, J_1 = 3.0, J_2 = 9.0 Hz, 1 H, ArH), 7.04 (d, J = 9.0 Hz, 1 H, ArH), 7.19 (d, J = 3.0 Hz, 1 H, ArH), 7.35−7.42 (m, 5 H, ArH) ppm. ^{13}C NMR ($CDCl_3$): δ = 56.3, 70.7, 96.1, 113.6, 114.8, 118.1, 119.6, 127.3, 128.1, 128.5, 136.5, 148.3, 154.1 ppm.

4-Benzyloxy-1-methoxymethoxy-2-(D_3)methylbenzene (7) from 9e: A solution of *n*BuLi (1.9 mL, 4.2 mmol, 2.2 M in hexane) was added to a solution of **9e** (970 mg, 3 mmol) in dry THF (15 mL) at −78 °C. Immediately afterwards a solution of CD_3I (1.12 mL, 18 mmol, 6 equiv.) in dry THF (10 mL) was added. After 2 h, GC analysis showed a 97% conversion of the starting material and formation of **7** in 90% yield.

4-Benzyloxy-(2-D_3)methylphenol (1) from 7: The crude mixture of **7** was dissolved in MeOH (25 mL), a catalytic amount of *p*-TsOH was added and the reaction was heated to 50 °C for 3 h. After evaporating the solvent, the residue was purified by column chromatography (Hex/EtOAc, 5:1) to afford **1** in 82% yield from **9e**.

4-Benzyloxy-(2-D_3)methylphenol (1) from 3: $ClCO_2Et$ (1.08 mL, 11.3 mmol, 2.6 equiv.) was added to a solution of **3** (1.06 g, 4.3 mmol) and Et_3N (1.6 mL, 11.3 mmol, 2.6 equiv.) in THF (45 mL) at 0 °C and the mixture stirred for 3 h at this temperature. The resulting white precipitate was filtered off and washed with THF (15 mL). The combined filtrates were concentrated to small volume, re-diluted with THF (15 mL) and carefully added to a solution of $NaBD_4$ (1.32 g, 34.7 mmol, 8 equiv.) in D_2O (15 mL) at 0 °C. The white suspension was allowed to warm to room temp. and stirred overnight. TLC (Hex/EtOAc/AcOH, 3:1:0.005) showed complete conversion of the starting acid. The mixture was neutralized with 1 M HCl and the THF evaporated off. The aqueous layer was extracted with Et_2O (3 × 100 mL), and the combined organic extracts were subsequently washed to neutrality with water

and dried over Na_2SO_4. The solution was concentrated in vacuo to give **1** (840 mg, 89% yield) as a pale-yellow solid. M.p. 85−88 °C. ^1H NMR ($CDCl_3$/TMS): δ = 4.55 (br. s, 1 H, OH), 4.99 (s, 2 H, $PhCH_2$), 6.68 (m, 2 H, ArH), 6.78 (m, 1 H, ArH), 7.35−7.45 (m, 5 H, ArH) ppm. ^2H NMR (CH_3Cl): δ = 2.2 (s, $ArCD_3$) ppm. ^{13}C NMR ($CDCl_3$): δ = 16.3, 71.0, 113.3, 115.7, 118.1, 125.2, 127.7, 128.1, 128.8, 137.2, 148.3 ppm. APCI-MS (in MeOH): *m*/*z* = 218 [M + H^+]. $C_{14}H_{11}D_3O_2$ (217.3): calcd. C 77.39, H 7.88; found C 77.18, H 7.65; 98% isotope purity by GC mass spectrometry.

6-*O*-Benzyl-(8-D_3)-(*all-rac*)-δ-tocopherol (11): A solution of isophytol (0.48 mL, 1.37 mmol) in *i*BuOAc (5 mL) was added dropwise very slowly (over 3 h) to a stirred mixture of **1** (200 mg, 0.91 mmol), 0.5 mL of aq. HCl (>36.5%) and anhydrous $ZnCl_2$ (190 mg, 1.37 mmol, 1.5 equiv.) in *i*BuOAc (5 mL) at 50 °C. 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 M HCl, and the MeOH evaporated off. Water (10 mL) was added and the mixture extracted with Et_2O (3 × 30 mL). The combined organic extracts were subsequently washed to neutrality with water, dried over Na_2SO_4 and concentrated to dryness. After purification by flash chromatography (Hex/EtOAc, 9:1), **11** (320 mg, 72% yield) was obtained as a brown-yellow oil. ^1H NMR ($CDCl_3$/TMS): δ = 0.7−1.9 [m, 38 H, C(3)H_2, C(2a)H_3 and $C_{16}H_{33}$ chain], 2.7 [t, *J* = 7.0, 2 H, C(4)H_2], 5.0 (s, 2 H, $PhCH_2$), 6.5 (d, *J* = 3.1 Hz, 1 H, ArH), 6.7 (d, *J* = 3.2 Hz, 1 H, ArH), 7.3−7.5 (m, 5 H, ArH) ppm. ^2H NMR ($CHCl_3$): δ = 2.11 [s, C(8)D_3] ppm. APCI-MS (in MeOH): *m*/*z* = 497 [M + 1].

(8-D_3)-(*all-rac*)-δ-Tocopherol (12): A suspension of **11** (150 mg, 0.3 mmol) and 5% palladium on carbon (160 mg) in EtOAc (6 mL) was stirred overnight under an atmosphere of hydrogen. After filtering through celite, the filtrate was concentrated in vacuo affording **12** (120 mg, ≈100% yield) as a pale-yellow dense liquid. ^1H NMR ($CDCl_3$/TMS): δ = 0.7−1.9 [m, 38 H, C(3)H_2, C(2a)H_3 and $C_{16}H_{33}$ chain], 2.65 [t, *J* = 7.0, 2 H, C(4)H_2], 4.4 (br. s, 1 H, OH), 6.38 [d, *J* = 2.6 Hz, 1 H, C(5)H], 6.47 [s, 1 H, C(7)H] ppm; no aromatic 8-methyl proton signal at δ = 2.09 ppm was detectable. ^2H NMR ($CHCl_3$): δ = 2.08 [s, C(8)D_3] ppm. ^{13}C NMR ($CDCl_3$): δ = 147.8, 146.0, 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. Isotope purity of 98% by GC-MS (TMS derivative).

6-*O*-Acetyl-5-formyl-(2*R*,4′*R*,8′*R*)-γ-tocopherol (14): NMO (1.14 g, 9.7 mmol, 4 equiv.) was added to a solution of **13**[15] (1.34 g, 2.41 mmol) in dry acetonitrile (20 mL). After stirring for 5 h at room temp., the solvent was evaporated and the crude residue purified by column chromatography (Hex/EtOAc, 15:1) to afford **14** (1.17 g, 92% yield) as a dense, yellow oil. ^1H NMR ($CDCl_3$/TMS): δ = 0.95−1.6 [m, 36 H, C(2a)H_3 and $C_{16}H_{33}$ chain], 1.7 (m, 2 H, $ArCH_2CH_2$), 2.02 (s, 3 H, $ArCH_3$), 2.1 (s, 3 H, $ArCH_3$), 2.3 (s, 3 H, CH_3CO), 3.05 (t, *J* = 6.2 Hz, 2 H, $ArCH_2CH_2$), 10.26 (s, 1 H, ArCHO) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.9, 13.0, 19.6, 19.7, 20.4, 20.9, 22.5, 22.6, 23.8, 24.7, 27.9, 30.5, 30.7, 32.6, 32.7, 37.2, 37.3, 37.4, 39.3. 39.9, 75.8, 120.5, 122.8, 128.2, 136.6, 145.0, 149.8, 169.6, 189.9 ppm.

6-*O*-Acetyl-(2*R*,4′*R*,8′*R*)-γ-tocopherol-5-carboxylic Acid (15): NH_2SO_3H (160 mg. 1.6 mmol, 1.6 equiv.) and water (7 mL) were added to a solution of **14** (490 mg, 1 mmol) in 1,4-dioxane (20 mL). After stirring for 20 min, $NaClO_2$ (180 mg, 1.4 mmol, 1.4 equiv.) and water (5 mL) were added. After stirring for a further 30 min, Na_2SO_3 (150 mg) was added to destroy the unreacted $NaClO_2$ and any HOCl formed during the reaction. Water was then added and the aqueous phase was extracted with Et_2O (3 × 50 mL). The combined organic extracts were subsequently washed to neutrality with water, dried over Na_2SO_4 and concentrated to dryness to give pure **15** (485 mg, 96% yield), without further purification, as a yellow oil. ^1H NMR ($CDCl_3$/TMS): δ = 0.95−1.6 [m, 36 H, C(2a)H_3 and $C_{16}H_{33}$ chain], 1.7 (m, 2 H, $ArCH_2CH_2$), 2.01 (s, 3 H, $ArCH_3$), 2.1 (s, 3 H, $ArCH_3$), 2.28 (s, 3 H, CH_3CO), 2.9 (t, *J* = 6.2 Hz, 2 H, $ArCH_2CH_2$), 9.2 (br. s, 1 H, $ArCO_2H$) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.8, 12.9, 19.6, 19.7, 20.6, 21.1, 22.5, 22.7, 24, 24.4, 24.7, 27.9, 30.6, 32.7, 37.2, 37.3, 39.3. 40.1, 76, 118.3, 121.5, 128.9, 130.3, 140.5, 150, 170.1, 171.9 ppm.

(2*R*,4′*R*,8′*R*)-γ-Tocopherol-5-carboxylic Acid (16): A solution of KOH in MeOH (10 mL, 2 M) was added to **15** (490 mg, 0.97 mmol). The solution was heated to 50 °C for 2 h, then the MeOH was evaporated off and water added. The aqueous phase was extracted with Et_2O (3 × 50 mL). The combined organic extracts were subsequently washed to neutrality with water, dried over Na_2SO_4 and concentrated to dryness to give pure **16** (420 mg, 93% yield), without further purification, as a yellow semi-solid. ^1H NMR ($CDCl_3$/TMS): δ = 0.95−1.6 [m, 36 H, C(2a)H_3 and $C_{16}H_{33}$ chain], 1.7 (m, 2 H, $ArCH_2CH_2$), 2.01 (s, 3 H, $ArCH_3$), 2.1 (s, 3 H, $ArCH_3$), 3.05 (t, *J* = 6.2 Hz, 2 H, $ArCH_2CH_2$), 9.8 (br. s, 1 H, ArOH), 11.1 (br. s, 1 H, $ArCO_2H$) ppm. ^{13}C NMR ($CDCl_3$): δ = 11.8, 13.0, 19.6, 19.7, 20.9, 22.6, 22.7, 23.6, 24, 24.4, 24.8, 27.9, 30.9, 31.4, 32.6, 32.8, 37.2, 37.3, 37.4, 39.3. 39.6, 74.6, 106.7, 119, 124, 136.1, 144.8, 155.9, 176.8 ppm.

(2*R*,4′*R*,8′*R*)-(5-D_3)-(2*R*,4′*R*,8′*R*)-α-tocopherol (17): $ClCO_2Et$ (0.22 mL, 2.26 mmol, 2.6 equiv.) was added to a solution of **16** (400 mg, 0.87 mmol) and Et_3N (0.32 mL, 2.26 mmol, 2.6 equiv.) in THF (10 mL) at 0 °C and the mixture stirred for 3 h at 0 °C. The resulting white precipitate was filtered off and washed with THF (10 mL). The combined filtrates were concentrated to small volume, re-diluted with THF (10 mL) and carefully added to a solution of $NaBD_4$ (295 mg, 6.97 mmol, 8 equiv.) in D_2O (10 mL) at 0 °C. The white suspension was allowed to warm to room temp. and stirred overnight. TLC (Hex/EtOAc/AcOH, 3:1:0.005) showed complete conversion of the starting acid. The mixture was neutralized with 1 M HCl and the THF evaporated off. The aqueous layer was extracted with Et_2O (3 × 30 mL), and the combined organic extracts were subsequently washed to neutrality with water and dried over Na_2SO_4. The solution was concentrated in vacuo, giving **17** (270 mg, 72% yield) as yellow oil. ^1H NMR ($CDCl_3$/TMS): δ = 0.7−1.9 [m, 38 H, C(3)H_2, C(2a)H_3 and $C_{16}H_{33}$ chain], 2.11 and 2.18 [s, 3 H, C(8)CH_3 and s, 3 H, CH_3C(7)], 2.6 [t, *J* = 7.0 Hz, 2 H, C(4)H_2], 4.2 (br. s, 1 H, OH) ppm. ^2H NMR ($CHCl_3$): δ = 2.08 [s, C(5)CD_3] ppm. ^{13}C NMR ($CDCl_3$): δ = 146.1, 145, 122.9, 121.3, 119.2, 117.3, 74.8, 40.3, 39.5, 37.8−38.1, 33.2, 33.1, 32.1, 28.4, 25.2, 24.9, 24.4, 23.2, 23.1, 21.8, 21.2, 20.4−20, 12.8, 12.2 ppm. APCI-MS (positive ion mode; in MeOH): *m*/*z* = 433 [M^+]. Isotope purity of 98.5% by GC-MS (TMS derivative). Optical purity assessed by chiral HPLC.

Acknowledgments

The University of Pisa and Centro di Eccellenza AMBISEN are acknowledged for financial support.

[1] IUPAC-IUB, *Eur. J. Biochem.* **1982**, *123*, 473−475.
[2] Q. Jiang, S. Christen, M. K. Shigenaga, B. N. Ames, *Am. J. Clin. Nutr.* **2001**, *74*, 714−722.
[3] H. J. Kayden, M. G. Traber, *J. Lipid Res.* **1993**, *34*, 343−358.
[4] C. Lauridsen, S. W. Leonard, D. A. Griffin, D. C. Liebler, T. D. McClure, M. G. Traber, *Anal. Biochem.* **2001**, *289*, 89−95.
[5] P. Mottier, E. Gremaud, P. A. Guy, R. J. Turesky, *Anal. Biochem.* **2002**, *301*, 128−135.
[6] R. Andreoli, P. Manini, D. Poli, E. Bergamaschi, A. Mutti, W. M. A. Niessen, *Anal. Biochem.* **2004**, *378*, 987−994.
[7] Th. Netscher, *Chimia* **1996**, *50*, 563−567 and references cited therein.
[8] F. Mazzini, E. Alpi, P. Salvadori, Th. Netscher, *Eur. J. Org. Chem.* **2003**, *2003*, 2840−2844.
[9] P. Mamalis, J. Green, S. Marcinkiewicz, D. McHale, *J. Chem. Soc.* **1959**, 3350−3357.
[10] M. Versteeg, B. Benzuidehoudt, D. Ferreira, *Heterocycles* **1993**, *36*, 1743−1746.
[11] V. Snieckus, *Chem. Rev.* **1990**, *90*, 879−933.
[12] R. Kranich, K. Eis, O. Geis, S. Mühle, J. W. Bats, H. Schmalz, *Chem. Eur. J.* **2000**, *6*, 2874−2894.
[13] N. Minami, S. Kijima, *Chem. Pharm. Bull.* **1979**, *27*, 816−820.
[14] D. Mitchell, C. W. Doecke, L. A. Hay, Th. M. Koenig, D. D. Wirth, *Tetrahedron Lett.* **1995**, *36*, 5335−5338.
[15] Th. Rosenau, W. D. Habicher, *Synlett* **1997**, *2*, 208−209.
[16] Th. Rosenau, C. Adelwöhrer, A. Hofinger, K. Mereiter, P. Kosma, *Eur. J. Org. Chem.* **2004**, *2004*, 1323−1329.
[17] Th. Rosenau, W. D. Habicher, *Tetrahedron* **1995**, *51*, 7919−7926.
[18] Th. Rosenau, A. Potthast, T. Elder, P. Kosma, *Org. Lett.* **2002**, *4*, 4285−4288.
[19] D. J. Dodsworth, M.-P. Calcagno, E. U. Ehrmann, *J. Chem. Soc., Perkin Trans. 1* **1981**, 2120−2124.

Received July 28, 2004

