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Vitamin E Metabolites: Synthesis of [D_2]- and [D_3]-γ-CEHC

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Deuterated analogues of α- and γ-CEHC, main urinary and plasma metabolites of vitamin E, can be traced and accurately determined quantitatively by MS in complex matrices. In that regard, here we report the first synthesis of *rac*-[D_3]-γ-CEHC together with a simple route to 7a,8a-[D_2]-γ-CEHC through the set up of efficient procedures for the preparation of the corresponding deuterated hydroquinone building blocks.

Introduction

Vitamin E is a generic term for 8 tocol-related vitamers (4 tocopherols and 4 tocotrienols) which together qualitatively exhibit the biological activity of α-tocopherol,[1] and they are essential micronutrients of plant origin that are involved in various biological processes that are relevant to human health and diseases.[2] Vitamers in each group differ in the number and position of methyl groups on the chroman ring (identified with the greek letters α-, β-, γ- and δ). The presence of unsaturation in the 3′-, 7′- and 11′-positions of the aliphatic side chain differentiates tocotrienols from tocopherols, while the phenolic group on the ring provides the classical lipoperoxyl radical scavenging activity of vitamin E molecules. However, it has now become clear that the functions of vitamin E far exceed those of a simple antioxidant.[3a–3c] The main catabolic route of vitamin E was disclosed in hepatic cells and applies to all forms of vitamin E (Figure 1).[4,5] It consists of side chain degradation initiated by a cytochrome P450 enzyme-catalyzed ω-hydroxylation, which is followed by a β-oxidation-like process. The main metabolites formed are 2,5,7,8-tetramethyl- and 2,7,8-trimethyl-2-(2′-carboxyethyl)-6-hydroxychromans, α-[6] and γ-CEHC,[7] respectively. These are excreted as glucuronide conjugates (in a lesser extent as sulfate conjugates) with the bile or urine. Plasma and urine contain more γ- than α-CEHC.[8] Interestingly, γ-CEHC is a natriuretic,[7] but α-CEHC is not.

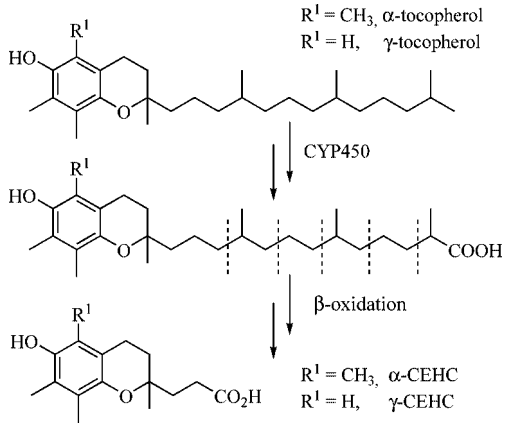

Figure 1. α- and γ-CEHC formation from α- and γ-tocopherol by cytochrome P450 Cyp4F2 enzyme followed by β-oxidation.[4]

Though the identification of CEHCs[6,7] as tocopherol and tocotrienol metabolites has stimulated a renewed interest in vitamin E biological properties, the role(s) of CEHCs in vitamin E metabolism is only partially understood, particularly in extra-hepatic tissues. Moreover, specific biological roles of CEHCs deserve further attention. The availability and utilization of stable isotope-labelled analogues greatly facilitates carrying out such studies.[2,9] They can be used as in vivo and in vitro probes and as internal standards for accurate quantitative determinations by mass spectrometry.[10,11]

In this context, here we describe the first synthesis of racemic [D_3]-γ-CEHC **19** as well as the preparation of [D_2]-γ-CEHC **8** by simple routes from the corresponding deuterated dimethylhydroquinones building blocks.

Results and Discussion

In 1996, Wechter et al.[7] reported the isolation and identification of a new natriuretic, γ-CEHC. Three years later,

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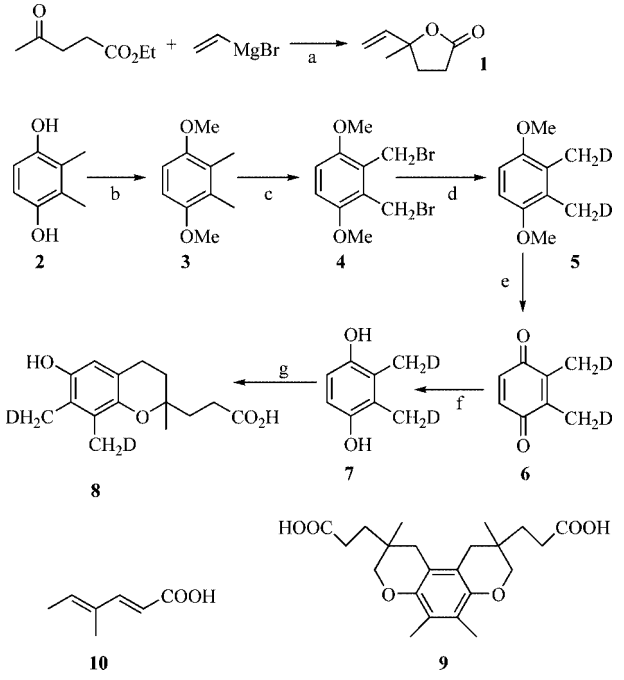
Swanson et al.[12] published the first synthesis of a [D_2]-γ-CEHC derivative, in which racemic γ-CECH, prepared by the condensation of 2,3-dimethylhydroquinone (DMHQ) and γ-methyl-γ-vinylbutyrolactone **1** (Scheme 1) in the presence of BF_3·Et_2O, was acetylated and dehydrogenated with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ). After deacetylation, the resulting 3,4-double bond was then reduced by Pd/C catalyzed D_2 deuteration, by analogy with the [D_2]-γ-tocopherol synthesis previously reported by Ingold et al.[13] The overall yield starting from DMHQ was 20% over five steps. Reduction of the tocopherol chromene 3,4-double bond with a heterogeneous catalyst and D_2 was investigated by Atkinson,[14] who reported various degrees of deuterium incorporation depending on the catalyst, substrate to catalyst ratio, concentration, temperature and solvent used. To avoid those problems, we took advantage of our experience in deuterated hydroquinone syntheses;[15a–15c] we used a different approach for the preparation of a [D_2]-γ-CEHC derivative. The aromatic methyl groups of dimethoxy derivative **3**, easily obtained from DMHQ, were monobrominated by classic NBS radical bromination.[16] Deuterium was then introduced by refluxing **4** with $LiAlD_4$ in THF, as the reaction was slow at room temperature. Ceric ammonium nitrate (CAN) oxidative demethylation in CH_3CN/H_2O followed by $NaBH_4$ reduction in $MeOH/H_2O$ at 0 °C smoothly afforded desired [D_2]-hydroquinone **7**. Both these two last reactions are very fast and give complete conversion in 10–20 minutes. Reduction by $NaBH_4$ in EtOH at room temperature proved to be less effective (70% yield).



Scheme 1. a) THF, 2 h. 57%; b) NaH, DMF then MeI, 4 h, 93%; c) NBS, $(Bz)_2O_2$, CCl_4, reflux, 3 h, 78%; d) $LiAlD_4$, THF, reflux, 3 h, 88%; e) CAN, CH_3CN/H_2O, 20 min, 88%; f) $NaBH_4$, $MeOH/H_2O$, 0 °C, 20 min, 93%; g) **1**, $ZnCl_2$, cat. HCl_{conc}, 1,4-dioxane, reflux 2 h, 42%.

The final condensation between DMHQ and lactone **1** to give γ-CEHC was the yield-limiting step. According to Kantoci et al.,[17] this acid catalyzed condensation leads to a mixture of γ-CEHC and double-alkylated product **9** (Scheme 1), the latter being the major product, as well as polymeric and oxidative byproducts even with careful exclusion of oxygen. After optimization of the reaction time, temperature and order of reagent addition, they managed to improve yields from 5 to 52% after crystallization on a 10 mmol scale (1 equiv. DMHQ, 2 equiv. BF_3·Et_2O, refluxing 1,4-dioxane, addition of 1.5 equiv. lactone **1** over 1h, then reaction quenching). By considering the difficulties described by those authors, alternative acidic conditions were explored to try to increase the reaction selectivity and γ-CEHC yields. The use of HCO_2H both as a solvent and an acid reagent following the protocol described by Gloor et al.[18] gave γ-CEHC in yields not exceeding 25%. As Amberlyst-15 proved to be rather effective in the analogous reaction between isophytol and trimethylhydroquinone in α-tocopherol synthesis,[19] it was tested in both catalytic and stoichiometric amounts. The use of 20 mol-% of the acid resin in refluxing dioxane gave poor conversion of DMHQ even after prolonged heating (10 h). Better results were obtained with a stoichiometric amount of Amberlyst-15 (1.5 equiv. of **1**), but conversion of DMHQ (50%) and yield of γ-CEHC (34%), calculated on the basis of the recovered hydroquinone and products at the end of reaction after column chromatography, were still unsatisfactory after 8 h, whereas the products selectivity was better than BF_3·Et_2O, with a 75:25 γ-CEHC/**9** ratio. Significant amounts of dienoic acids (20–30% of starting lactone **1**) were isolated by chromatography; acid **10** was the major product, according to the reported data.[20] Hence, the low conversion that was observed can be the result of the slow reaction of lactone **1** with DMHQ in those conditions, which allows for the competitive transformation of **1** to dienoic acids and, therefore, the consequent negative impact on the yield.

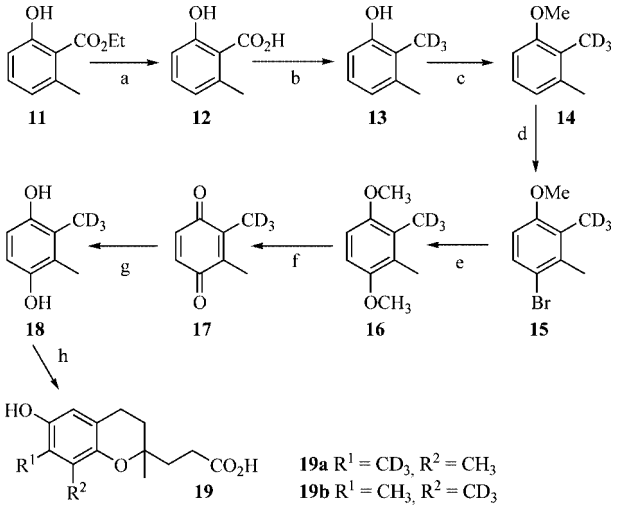
Anhydrous $ZnCl_2$ was efficiently used in the synthesis of tocopherols,[15a,19,21] often in the presence of catalytic amounts of concentrated HCl to increase its acidity. Trials carried out with $ZnCl_2$ alone in 1,4-dioxane (2 equiv., 1.5 equiv. of **1**) showed very low conversion after prolonged heating. The addition of 20 mol-% of concentrated HCl was effective even at room temperature, with a 50% conversion of DMHQ and 60:40 γ-CEHC/**9** ratio of the products. Heating the reaction at reflux for 2 h led to the complete disappearance of the starting lactone (1.5 equiv., addition over 1 h), with the recovery of 20–30% of dienoic acids, 27% of unreacted DMHQ, 43% of γ-CEHC and 30% of **9**. An increase in the amount of HCl gave a higher percentage of **9** and dienoic acids, as expected. An experiment was also carried out with the use of a lower amount of **1** (1.2 equiv., addition over 1 h) heated at reflux in dioxane for 4 h. The conversion of DMHQ was lower (65%), as well as the yield of γ-CEHC (34%), but the γ-CEHC/**9** ratio was better (70:30).

With the data collected taken into consideration, we preferred to obtain partial conversion of labelled **7** to desired

compound **8** but with a lower transformation into the dialkylated byproduct and recovery of unreacted **7** than complete conversion of **7** accompanied by the formation of labelled **9** as the major product, as reported with the use of $BF_3{\cdot}Et_2O$. Hence, we obtained $[D_2]$-γ-CEHC **8** in 42% yield and high isotope purity (97.7%), 21.9% overall yield from **2** over six steps with the use of $ZnCl_2$ and 20 mol-% HCl and 1.5 equiv. of lactone **1**. Interestingly, the same reaction conditions applied in the condensation of trimethylhydroquinone and **1** afforded α-CEHC in 75% yield.

Labelled analogues with a molecular weight increased by at least three mass units are generally preferred to obtain accurate determinations by MS/MS analyses in order to avoid interference of natural isotopes of the analyte with the *m*/*z* value of the labelled compound.[22] Therefore, we also devised a synthetic route to $[D_3]$-γ-CEHC **19** (Scheme 2). Saponification of commercially available ester **11** by KOH in $MeOH/H_2O$ gave corresponding acid **12** almost quantitatively. The established procedure for the reduction of salicylic acids to 2-methylphenols by $ClCO_2Et$-$NaBD_4$ [23] was also successful in the conversion of acid **12** to trideuterated dimethyl phenol **13**. According to this method, the reaction of **12** with $ClCO_2Et$ gave the corresponding bis(ethoxycarbonyl) derivative, whose treatment with $NaBD_4$ in D_2O/THF directly provided $[D_3]$-dimethylphenol **13** in 71% yield. Preparation of DMHQ from 2,3-dimethylphenol is generally performed through the oxidation of 2,3-dimethylphenol to the corresponding 1,4-benzoquinone by strong oxidants and transition metals[24] or by coupling with diazotized sulfanilic acid followed by reduction–oxidation steps,[16] usually in only fair yields. We were attracted by the procedure described for the multigram-scale synthesis of 2,3-bis(bromomethyl)-1,4-dimethoxybenzene,[16] in which the key step is the high-yielding nucleophilic substitution of bromide in 4-bromo-2,3-dimethylmethoxybenzene with methoxide catalyzed by CuI. Therefore, **13** was transformed into corresponding methyl ether **14** and then monobrominated with benzyltrimethylammonium tribromide[25] ($BTMABr_3$) to afford *para* isomer **15** exclusively. Conversely, attempts to insert the bromine atom at acid **12** stage under the same reaction conditions gave a 30:70 *ortho*/*para* mixture of isomers. The subsequent nucleophilic substitution of **15** with $NaOCH_3$ in DMF/MeOH in the presence of 15 mol-% CuI occurred smoothly when heated at reflux for 7 h to provide dimethoxy derivative **18** in 89% yield. By applying the same oxidative demethylation-reduction sequence used for the preparation of **7**, $[D_3]$-DMHQ **18** was obtained in 80% yield from **16**.

The subsequent acid catalyzed condensation of **18** with lactone **1** as described above for **8** provided $[D_3]$-γ-CEHC **19** as a mixture of 7- and 8-CD_3 isomers in 43% yield, 16.5% overall yield from **11** over eight steps.



Scheme 2. a) KOH, $H_2O/MeOH$, 2:1, 50 °C, 6 h, 94%; b) $ClCO_2Et$, Et_3N, 0 °C, 2 h, then $NaBD_4$, 8 equiv., D_2O/THF, 0 °C, 3 h, 71%; c) NaH, DMF then MeI, 3 h, 89%; d) $BTMABr_3$, $CH_2Cl_2/MeOH$, 1.5 h, 92%; e) $NaOCH_3$, CuI, MeOH-DMF, reflux, 7 h, 88%; f) CAN, CH_3CN/H_2O, 20 min, 88%; g) $NaBH_4$, $MeOH/H_2O$, 0 °C, 20 min, 91%; h) **1**, $ZnCl_2$, cat. HCl_{conc}, 1,4-dioxane, reflux 2 h, 43%.

Conclusions

We described an approach different to the one previously reported[12] for the synthesis of a $[D_2]$-γ-CEHC derivative, through the efficient preparation of deuterated hydroquinone **7** that was subsequently condensed with methyl-γ-vinylbutyrolactone in the presence of $ZnCl_2$ and HCl to provide desired $[D_2]$-γ-CEHC **8**. Moreover, we report the first synthesis of $[D_3]$-γ-CEHC **19** starting from trideuterated hydroquinone **18**, through a smooth procedure to provide needed labelled hydroquinone **18** in a plain and easy way. High isotopic purity was obtained in both cases, which makes the two labelled metabolites suitable as internal standards for quantitative determinations by mass spectrometry. Further studies are in progress regarding the possibility of improving the acid catalyzed condensation step through the optimization of the reaction parameters and the set up of a convenient procedure for the monoprotection of one of the two phenolic groups of DMHQ, to avoid the formation of dialkylated **9**. It should be noted that hydroquinones **7** and **18** can also be employed as building blocks for the synthesis of labelled racemic γ-tocotrienols.

Experimental Section

All reactions were performed under an inert atmosphere (Ar). ^1H,^2H and ^{13}C NMR spectra were recorded with a Varian VXR 300 spectrometer with Me_4Si as internal standard (^1H- and ^{13}C NMR) or CD_3OD as internal standard (^2H NMR). GC–MS analyses and deuteration level was determined by GC–MS, also taking into account the ionization pattern of the corresponding unlabeled material, with a 6890N GC system (Agilent Technologies) coupled with a 5973 mass selective detector (Agilent Technologies) single quadrupole mass spectrometer with an HP-5MS capillary column (30m × 0.25 mm, 0.25 μm film thickness). Operating conditions: injector temperature 270 °C; oven program temperature 2 min, 80 °C, increased at 30°/min to 280 °C, 2 min; transfer line temperature 280 °C; the MS ion source temperature was kept at 230 °C and the

MS quadrupole temperature at 150 °C. Deuteration level of **8** and **19** was determined by LC–MS with an Applied Biosystem API 4000 mass spectrometer. Commercial reagents and solvents were purchased from Aldrich or Fluka and purified by standard methods when necessary. Ethyl 6-methylsalicylate was purchased from TCI Europe N.V. (Haven, Belgium). γ-Methyl-γ-vinylbutyrolactone was prepared as described by Wechter et al.[7] THF was distilled from Na/K alloy, 1,4-dioxane from CaH_2. All other commercial reagents were used without further purification. Column and flash chromatography were performed on silica gel 60 (70–230 mesh and 230–400 mesh). TLC was performed with silica gel Macherey–Nagel Alugram Sil G/UV_{254} (0.20 mm). Work up involved the addition of water and three extractions into the solvent specified. The organic extracts were combined, washed with water and $NaCl_{aq}$ until the aqueous phase was neutral, dried with 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.

1,4-Dimethoxy-2,3-dimethylbenzene (3): To a stirred suspension of dry NaH (300 mg, 12 mmol, 2.4 equiv.) in dry DMF (8 mL) was added a solution of hydroquinone **2** (690 mg, 5 mmol) in dry DMF (6 mL). The reaction mixture was stirred for 40 min and then cooled to 0 °C. A solution of CH_3I (0.76 mL, 12 mmol, 2.4 equiv.) in dry DMF (4 mL) was then added dropwise. The reaction mixture was warmed to room temp. and stirred for 3 h. The end of the reaction was confirmed by TLC (Hex/EtOAc, 5:1). Water (10 mL) was added, and after the usual work, up in Et_2O, pure **3** (770 mg, 93% yield) was obtained as a white–brown solid. M.p. 78–79 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.17 (s, 6 H), 3.78 (s, 6 H), 6.66 (s, 2 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 12.2, 56.2, 108.0, 126.8, 152.2 ppm.

2,3-Bis(bromomethyl)-1,4-dimethoxybenzene (4): Compound **3** (670 mg, 4 mmol) was dissolved in a CCl_4 (15 mL) solution of benzoyl peroxide (100 mg, 0.4 mmol, 0.1 equiv.). The solution was heated at reflux for 2.5 h, cooled to room temp., filtered and the residue washed with CCl_4. The filtrate was then concentrated under vacuum, and the residue was purified by column chromatography (Hex/EtOAc, 10:1) to afford **4** (1.02 g, 78% yield) as a pale yellow solid. M.p. 150–152 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.86 (s, 6 H), 4.75 (s, 4 H), 6.85 (s, 2 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 23.8, 56.2, 112.1, 126.3, 151.7 ppm.

1,4-Dimethoxy-2,3-bis[($^2H_1$)methyl]benzene (5): To a stirred suspension of $LiAlD_4$ (330 mg, 7.7 mmol, 2.5 equiv.) in dry THF (8 mL), a solution of dibromide **4** (1.0 g, 3.1 mmol) in dry THF (10 mL) was added dropwise. The reaction mixture was heated at reflux for 3 h. The end of the reaction was confirmed by TLC (Hex/EtOAc, 10:1). The mixture was cooled to room temp., water was added and then made acidic with HCl (1 M). THF was distilled off and, after the usual work up in Et_2O, the residue was purified by column chromatography (Hex/EtOAc, 10:1) to afford **5** (460 mg, 88% yield) as a white solid. M.p. 80–82 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.17 (t, J = 2.1 Hz, 4 H), 3.80 (s, 6 H), 6.68 (s, 2 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 12.0 (t, J = 19.4 Hz), 56.3, 108.1, 126.9, 152.1 ppm. GC–MS (EI): m/z (%) = 168 (88), 169 (11) 153 (100). $C_{10}H_{12}D_2O_2$ (168.2): calcd. C 71.39, H 9.58; found C 71.27, H 9.55. Isotope purity of 97.7% by GC–MS (97.7% [D_2]-, 2.1% [D_1]- and 0.2% [D_0]-**5**).

2,3-Bis[($^2H_1$)methyl]-1,4-benzoquinone (6): A solution of CAN (3.1 g, 5.6 mmol, 2.2 equiv.) in H_2O (6 mL) was added to a stirred solution of **5** (420 mg, 2.5 mmol) in CH_3CN (5 mL). After 20 min, TLC (Hex/EtOAc, 10:1) showed complete conversion of the reagent. Water was added to the red solution, and after usual work-up in CH_2Cl_2, the residue was purified by passage through a short plug of silica gel (CH_2Cl_2) to afford pure **6** (300 mg, 88% yield) as a yellow solid. M.p. 55–57 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.97 (t, J = 1.7 Hz, 4 H), 6.68 (s, 2 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 12.1 (t, J = 19.9 Hz), 136.4, 141.2, 187.6 ppm. GC–MS (EI): m/z (%) = 138 (60), 139 (16), 110 (100). $C_8H_6D_2O_2$ (138.1): calcd. C 69.55, H 7.29; found C 69.35, H 7.27. Isotope purity of 97.7% by GC–MS. (97.7% [D_2]-, 2.1% [D_1]- and 0.2% [D_0]-**6**).

1,4-Dihydroxy-2,3-bis[($^2H_1$)methyl]benzene (7): $NaBH_4$ (60 mg, 1.5 mmol, 1.2 equiv.) was added to a solution of **6** (170 mg, 1.2 mmol) in MeOH/H_2O (5:1, 6 mL) at 0 °C. After 20 min, TLC (Hex/EtOAc, 3:1) showed complete conversion of the reagent. Water was added (4 mL), and the mixture was made acidic with HCl (1 M). After the usual work up in EtOAc, the residue was purified by passage through a short plug of silica gel (Hex/EtOAc, 3:1) to afford **7** (165 mg, 93% yield) as a white–brown solid. M.p. 224–227 °C. ^{1}H NMR (300 MHz, CD_3OD): δ = 2.07 (t, J = 2.1 Hz, 4 H), 4.83 (br. s, 2 H), 6.47 (s, 2 H) ppm. ^{13}C NMR (75 MHz, CD_3OD): δ = 12.9 (t, J = 19.4 Hz), 114.2, 126.1, 149.9 ppm. GC–MS (EI): m/z (%) = 140 (100), 124 (66). $C_8H_8D_2O_2$ (140.2): calcd. C 68.55, H 8.63; found C 68.65, H 8.64. Isotope purity of 97.7% by GC–MS (97.7% [D_2]-, 2.1% [D_1]- and 0.2% [D_0]-**7**).

Racemic 2-(2′-Carboxyethyl)-6-hydroxy-2-methyl-7,8-bis[($^2H_1$)methyl]-chroman (8): A solution of lactone **1** (190 mg, 1.5 mmol, 1.5 equiv.) in anhydrous 1,4-dioxane (2 mL) was added over 1 h to a stirred solution of **7** (140 mg, 1 mmol), anhydrous $ZnCl_2$ (280 mg, 2 mmol, 2 equiv.) and HCl_{conc} (0.01 mL) in anhydrous 1,4-dioxane (3 mL). The mixture was heated at reflux for 2 h. Dioxane was evaporated, water was added, and after the usual work up in EtOAc, the residue was purified by flash chromatography (Hex/EtOAc/AcOH, 2:1:0.003). The brown semisolid was recrystallized from Hex/Et_2O, 1:1 at 0 °C to afford **8** (110 mg, 42% yield) as a white solid. M.p. 145–149 °C. ^{1}H NMR (300 MHz, CD_3OD): δ = 1.21 (s, 3 H), 1.71–1.93 (m, 4 H), 2.05 (t, J = 2.2 Hz, 4 H), 2.44 (m, 2 H), 2.64 (m, 2 H), 4.91 (br. s, 2 H), 6.33 (s, 1 H) ppm. ^{2}H NMR (46 MHz, CH_3OH): δ = 2.06 (t, J = 2.2 Hz) ppm. ^{13}C NMR (75 MHz, CD_3OD): δ = 12.6 (m), 23.9, 24.6, 30.3, 33.5, 36.7, 76.2, 113.9, 119.7, 124.1, 127.1, 146.6, 149.7, 178.6 ppm. APCI-LC–MS (MeOH, negative ion mode): m/z = 265 [M – H]$^-$. $C_{15}H_{18}D_2O_4$ (266.3): calcd. C 67.65, H 8.32; found C 67.47, H 8.29. Isotope purity of 97.7% by LC–MS (97.7% [D_2]-, 2.1% [D_1]- and 0.2% [D_0]-**8**).

6-Methylsalicylic Acid (12): Ethyl 6-methylsalicylate **11** (2.99 g, 16.6 mmol) was dissolved in a H_2O/MeOH, 2:1 solution of KOH (40 mL, 3.5 M). The resulting solution was heated at 50 °C for 6 h, cooled to room temp. and then made acidic with HCl_{conc}. After work up in EtOAc, the residue obtained was purified by column chromatography (Hex/EtOAc, 5:1) to afford **12** (1.67 g, 93.2% yield) as a white solid. M.p. 173–175 °C. ^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD): δ = 2.52 (s, 3 H), 4.82 (br. s, 2 H), 6.70 (d, J = 7.5 Hz, 1 H), 6.76 (d, J = 7.8 Hz, 1 H), 7.23 (t, J = 7.9 Hz, 1 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$/CD_3OD): δ = 23.8, 112.9, 115.2, 123.0, 134.1, 142.4, 162.8, 174.2 ppm.

2-[($^2H_3$)Methyl]-3-methylphenol (13): To a solution of **12** (1.74 g, 11.4 mmol) and Et_3N (4.05 mL, 29 mmol, 2.5 equiv.) in THF (70 mL) at 0 °C, $ClCO_2Et$ (2.77 mL, 29 mmol, 2.5 equiv.) was added, and the mixture stirred for 2 h at 0 °C. The resulting white precipitate was filtered off and washed with THF (30 mL). The combined filtrates were concentrated to small volume, rediluted with THF (15 mL) and slowly added to a solution of $NaBD_4$ (3.8 g, 91.2 mmol, 8 equiv.) in D_2O (25 mL) and THF (10 mL) at 0 °C.

After 3 h at 0 °C, the white suspension was allowed to rise to room temp. and stirred overnight. The mixture was then made acidic with HCl_{conc} and THF evaporated. After work up in EtOAc, the residue was purified by column chromatography (CH_2Cl_2) to afford **13** (1.01 g, 71% yield) as a white crystalline solid. M.p. 72–75 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.28 (s, 3 H), 4.87 (br. s, 1 H), 6.63 (d, J = 8.1 Hz, 1 H), 6.77 (d, J = 7.2 Hz, 1 H), 6.98 (t, J = 7.7 Hz, 1 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 11.5 (m), 20.0, 112.6, 122.3, 122.4, 126.0, 138.3, 153.5 ppm. GC–MS (EI): *m*/*z* (%) = 125 (100), 110 (54). $C_8H_7D_3O$ (125.2): calcd. C 76.76, H 10.46; found C 76.85, H 10.44. Isotope purity of 99.0% by GC–MS (99.0% [D_3]-, 0.8% [D_2]- and 0.2% [D_1]-**13**).

1-Methoxy-2-[($^2H_3$)methyl]-3-methylbenzene (14): To a stirred suspension of dry NaH (150 mg, 6 mmol, 1.2 equiv.) in dry DMF (4 mL) was added a solution of phenol **13** (630 mg, 5 mmol) in dry DMF (6 mL). The reaction mixture was stirred for 40 min and then cooled to 0 °C. A solution of CH_3I (0.38 mL, 6 mmol, 1.2 equiv.) in dry DMF (4 mL) was then added dropwise. The reaction mixture was warmed to room temp. and stirred for 2 h. The end of the reaction was confirmed by TLC (Hex/EtOAc, 5:1). Water (10 mL) was added, and after usual work up in Et_2O, pure **14** (620 mg, 89% yield) was obtained as a nearly colourless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.37 (s, 3 H), 3.90 (s, 3 H), 6.81 (d, J = 8.4 Hz, 1 H), 6.87 (d, J = 8.7 Hz, 1 H), 7.16 (t, J = 7.8 Hz, 1 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 11.7 (m), 20.3, 55.7, 108.1, 122.5, 125.1, 126.1, 138.1, 157.9 ppm. GC–MS (EI): *m*/*z* (%) = 139 (100), 124 (79). $C_9H_9D_3O$ (139.2): calcd. C 77.65, H 10.86; found C 77.55, H 10.89. Isotope purity of 99.0% by GC–MS (99.0% [D_3]-, 0.8% [D_2]- and 0.2% [D_1]**14**).

4-Bromo-1-methoxy-2-[($^2H_3$)methyl]-3-methylbenzene (15): A solution of $BTMABr_3$ (1.45 g, 3.7 mmol) in CH_2Cl_2 (16 mL) was added dropwise to a stirred solution of **14** (510 mg, 3.7 mmol) in MeOH/CH_2Cl_2 (2:3, 25 mL). After 1.5 h, TLC (Hex) showed complete conversion of the reagent. $NaHSO_3$ (5%, 4 mL) was added, and the mixture was stirred for 15 min. Water was added, MeOH evaporated, and after the usual work up in Et_2O, the residue was purified by passage through a short plug of silica gel (Et_2O) to afford pure **15** (740 mg, 92% yield) as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.43 (s, 3 H), 3.85 (s, 3 H), 6.64 (d, J = 8.7 Hz, 1 H), 7.40 (d, J = 8.7 Hz, 1 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 11.9 (m), 19.8, 55.7, 109.4, 116.3, 126.8, 129.6, 136.8, 156.7 ppm. GC–MS (EI): *m*/*z* (%) = 217 (100), 219 (98), 202 (46), 204 (44). $C_9H_8D_3BrO$ (218.1): calcd. C 49.56, H 6.47, Br 36.64; found C 49.72, H 6.45, Br 36.57. Isotope purity of 99.0% by GC–MS (99.0% [D_3]-, 0.8% [D_2]- and 0.2% [D_1]-**15**).

1,4-Dimethoxy-2-[($^2H_3$)methyl]-3-methylbenzene (16): A solution of $NaOCH_3$ (4.37 M) in MeOH (2.5 mL) was added to a DMF solution (2.5 mL) of **15** (700 mg, 3.2 mmol) and CuI (100 mg, 0.5 mmol). The resulting solution was heated at reflux for 7 h when TLC (Hex) showed complete conversion of the reagent. An aqueous solution of NH_3 (5%) was then added to remove the copper catalyst, and after the usual work up in Et_2O, the residue was purified by passage through a short plug of silica gel (Hex) to afford pure **16** (470 mg, 88% yield) as a pale yellow solid. M.p. 76–78 °C ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.17 (s, 3 H), 3.78 (s, 6 H), 6.66 (s, 2 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 12.2 (m), 56.2, 108.0, 126.8, 126.9, 152.1 ppm. GC–MS (EI): *m*/*z* (%) = 169 (100), 154 (90). $C_{10}H_{11}D_3O_2$ (169.2): calcd. C 70.97, H 10.12; found C 70.81, H 10.08. Isotope purity of 99.0% by GC–MS (99.0% [D_3]-, 0.8% [D_2]- and 0.2% [D_1]-**16**).

2-[($^2H_3$)Methyl]-3-methyl-1,4-benzoquinone (17): A solution of CAN (2.78 g, 5.1 mmol, 2.2 equiv.) in H_2O (6 mL) was added to a stirred solution of **16** (400 mg, 2.3 mmol) in CH_3CN (6 mL). After 20 min, TLC (Hex/EtOAc, 10:1) showed complete conversion of the reagent. Water was added to the red solution, and after the usual work up in CH_2Cl_2, the residue was purified by passage through a short plug of silica gel (CH_2Cl_2) to afford pure **17** (280 mg, 88% yield) as a yellow solid. M.p. 57–59 °C ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.00 (s, 3 H), 6.67 (s, 2 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 12.3 (m), 136.4, 141.2, 187.5 ppm. GC–MS (EI): *m*/*z* (%) = 139 (56), 111 (100). $C_8H_5D_3O_2$ (139.2): calcd. C 69.04, H 7.96; found C 69.21, H 7.98. Isotope purity of 98.9% by GC–MS (98.9% [D_3]-, 0.9% [D_2]- and 0.2% [D_1]-**17**).

1,4-Dihydroxy-2-[($^2H_3$)methyl]-3-methylbenzene (18): $NaBH_4$ (80 mg, mmol, 1.2 equiv.) was added to a solution of **15** (250 mg, 1.7 mmol) in MeOH/H_2O (5:1, 6 mL) at 0 °C. After 20 min, TLC (Hex/EtOAc, 3:1) showed complete conversion of the reagent. Water was added, and the mixture was made acidic with HCl (1 M). After the usual work up in EtOAc, the residue was purified by passage through a short plug of silica gel (Hex/EtOAc, 3:1) to afford **18** (220 mg, 91% yield) as a white solid. M.p. 226–228 °C. ^{1}H NMR (300 MHz, CD_3OD): δ = 2.09 (s, 3 H), 4.86 (br. s, 2 H), 6.45 (s, 2 H) pm. ^{13}C NMR (75 MHz, CD_3OD): δ = 13.0 (m), 13.1, 114.1, 126.1, 1499 ppm. GC–MS (EI): *m*/*z* (%) = 141 (100), 125 (32). $C_8H_7D_3O_2$ (141.2): calcd. C 68.06, H 9.28; found C 68.21, H 9.26. Isotope purity of 98.9% by GC–MS (98.9% [D_3]-, 0.9% [D_2]- and 0.2% [D_1]-**18**).

Racemic 2-(2′-Carboxyethyl)-6-hydroxy-7-[($^2H_3$)Methyl]-2,8-dimethylchroman (19a) and 2-(2′-Carboxyethyl)-6-hydroxy-8-[($^2H_3$)-Methyl]-2,7-dimethylchroman (19b) Mixture: A solution of lactone **1** (250 mg, 2 mmol, 1.5 equiv.) in anhydrous 1,4-dioxane (3 mL) was added over 1 h to a stirred solution of **18** (190 mg, 1.35 mmol), anhydrous $ZnCl_2$ (370 mg, 2.7 mmol, 2 equiv.), HCl_{conc} (0.02 mL) in anhydrous 1,4-dioxane (3 mL). The mixture was heated at reflux for 2 h. Dioxane was evaporated, water was added, and after the usual work up in EtOAc, the residue was purified by flash chromatography (Hex/EtOAc/AcOH, 2:1:0.003). The brown semisolid was recrystallized from Hex/Et_2O, 1:1 at 0 °C to afford **19a+19b** (150 mg, 43% yield) as a white solid. According to the NMR peaks assignment reported by Wechter,[7] the mixture composition was calculated from ^{2}H NMR: 2.05 (8a CD_3, **19b**): 2.08 (7a CD_3, **19a**) peaks ratio, **19a/19b** = 53:47. M.p. 144–148 °C ^{1}H NMR (300 MHz, CD_3OD): δ = 1.20 (s, 3 H), 1.71–1.95 (m, 4 H), 2.04 and 2.06 (two singlets, their sum 3 H, $ArCH_3$ **19a** and **19b**, respectively), 2.43 (m, 2 H), 2.65 (m, 2 H), 4.91 (br. s, 2 H), 6.33 (s, 1 H) ppm. ^{2}H NMR (46 MHz, CH_3OH): δ = 2.05 (s), 2.08 (s) ppm. ^{13}C NMR (75 MHz, CD_3OD): δ = 12.7–12.8 (m), 24.0, 24.7, 30.4, 33.4, 36.7, 76.2, 113.9, 119.7, 124.1, 127.1, 146.6, 149.7, 178.6 ppm. APCI-LC–MS (MeOH, negative ion mode) *m*/*z* = 266 [M – H]$^-$. $C_{15}H_{17}D_3O_4$ (267.3): calcd. C 67.39, H 8.67; found C 67.15, H 8.63. Isotope purity of 98.9% by LC–MS (98.9% [D_3]-, 0.9% [D_2]- and 0.2% [D_1]-**19**).

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