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# First Synthesis of *rac*-(5-$^2H_3$)-α-CEHC, a Labeled Analogue of a Major Vitamin E Metabolite

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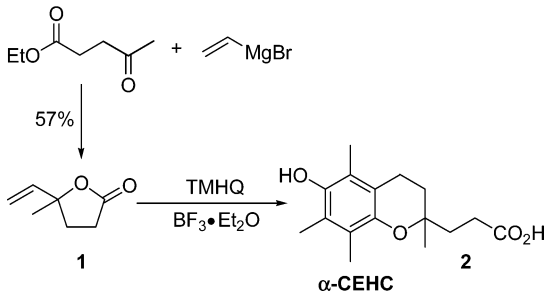
**Abstract:** Over the past few years, quantitative determination of α- and γ-CEHC, main urinary metabolites of vitamin E, has been becoming more and more important as a key parameter in assessing oxidative stress and supplementation of tocopherols. The use of deuterated analogues of these metabolites allows their accurate determination even in complex matrixes. While preparation of γ-CEHC-$d_2$ has already been described, here we report the first synthesis of α-CEHC-$d_3$ together with α-tocopheronolactone-$d_3$, its oxidation product.

Vitamin E is a generic term for tocopherols and tocotrienols that exhibit the biological activity of α-tocopherol.[1] Each group can occur in α-, β-, γ-, and δ-forms, which differ in the number and position of methyl groups on the chroman ring. Two of these homologues, α- and γ-tocopherol, are the most important ones for human nutrition, playing an essential role against oxidative stress and in preventing some chronic degenerative diseases.[2]

Over the past few years, there has been a growing and renewed interest in determining urinary metabolites of vitamin E because of their proposed relationship with oxidative stress and vitamin E supply.[3] Simon et al. were the first who isolated vitamin E-derived compounds from the urine of animals and humans supplied with large doses of α-tocopherol.[4] In these so-called Simon metabolites, the chroman ring has been opened and oxidized to the quinone structure (α-tocopheronolactone and α-tocopheronic acid). Recently, another α-tocopherol-related compound has been found[3] as the major urinary metabolite present in human urine, the 2,5,7,8-tetramethyl-2-(β-carboxyethyl)-6-hydroxychroman **2** (α-CEHC). Shortly afterward, also the γ-homologue (γ-CEHC) was isolated as a new natriuretic factor.[5] The identification of α-CEHC, in which the chroman ring is intact, led to the hypothesis that the previously identified Simon metabolites were artifactually produced by oxidation of α-CEHC during the extraction procedure. Therefore, confirmation of the authenticity and origin of the small amounts of α-tocopheronolactone observed in human urines is important to understand the utility of this metabolite as biomarkers of oxidative stress.[6]

**SCHEME 1. Preparation of Unlabeled α-CEHC**




In that regard, suitable analytical methods have to be developed to avoid oxidation of the metabolites during sample preparation necessary for the analysis. Recently, Muller et al. reported the first direct analysis of conjugated vitamin E metabolites using ESI-MS-MS,[7] showing a number of advantages over previous methods. However, they suggested the improvement of the method introducing a separation step (HPLC) prior to MS analysis together with the strong requirement of the use of vitamin E deuterated standards to allow more accurate quantitations.

Here we describe the first synthesis of racemic α-CEHC-$d_3$ **12** and its oxidation product, α-tocopheronolactone-$d_3$ **13**, through the setting up of an efficient route to the key building block trimethylhydroquinone-$d_3$ **11**.

Unlabeled α-CEHC was prepared in a satisfactory way by Kantoci et al.[8] by condensing trimethylhydroquinone (TMHQ) with γ-methyl-γ-vinylbutyrolactone (**1**) in the presence of $BF_3 \cdot Et_2O$.[9] The lactone can be easily obtained on a gram scale from inexpensive vinylmagnesium bromide and ethyl levulinate (Scheme 1).

As far as deuterated analogues are concerned, however, there are no reports regarding labeled α-CEHC and only one paper describing the synthesis of the analogue γ-CEHC-$d_2$ by Swanson et al.[10] The latter was prepared through DDQ dehydrogenation of the saturated ring on acetylated racemic γ-CEHC, followed by palladium catalyzed deuteration, with a 25% yield over five steps based on the starting dimethylhydroquinone.

For the synthesis of deuterated α-CEHC we used a different approch. In the preparation of labeled δ-toco-

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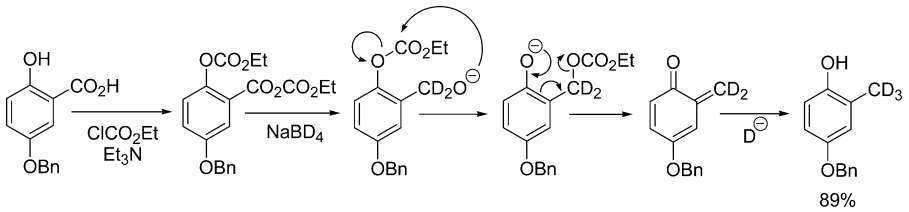
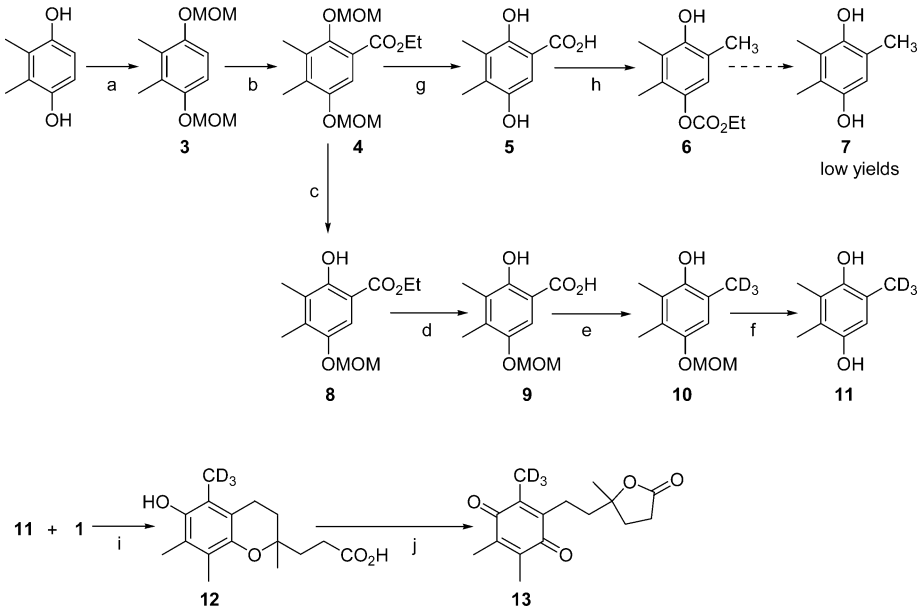
**SCHEME 2. Two-Step Reduction of 5-Benzyloxy-2-hydroxybenzoic Acid into 4-Benzyloxy-2-($^2H_3$)-methylphenol**




**SCHEME 3. Synthesis of α-CEHC-$d_3$ 12 and Lactone 13**[a]







[a] Key: (a) NaH, DMF, then MOMCl, 93%; (b) *t*-BuLi, hexane, $ClCO_2Et$, 0 °C, 84%; (c) $MgBr_2$, $C_6H_6$, rt, overnight, 91%; (d) KOH 2 M, MeOH, 50 °C, 2.5 h, then HCl pH = 4, quant; (e) (1) $ClCO_2Et$, $Et_3N$, 0 °C, 2 h, (2) $NaBD_4$, 8 equiv, $D_2O$/THF, 0 °C, 3 h, 89%; (f) *p*TsOH, MeOH, 50 °C, 3 h, 90%; (g) (1) KOH 4 M, EtOH/$H_2O$, (2) THF, $HCl_{conc}$, 86%; (h) (1) $ClCO_2Et$, $Et_3N$, 0 °C, 2 h, (2) $NaBH_4$, 8 equiv, $H_2O$/THF, 0 °C, 3 h, 70%; (i) dioxane, $BF_3{\cdot}Et_2O$, 110 °C, 5 h, 79%; (j) MeOH/$H_2O$, $FeCl_3{\cdot}6H_2O$, 82%.

pherol, we set up and optimized a two steps reduction procedure that afforded the synthesis of 4-benzyloxy-2-($^2H_3$)-methylphenol[11] in very good yield from 5-benzyloxy-2-hydroxybenzoic acid. According to this protocol, the starting hydroxybenzoic acid was quantitatively converted with ethyl chloroformate into a bis-ethoxycarbonyl derivative. Treatment with $NaBD_4$ in $D_2O$/THF solution directly afforded the labeled 2-methyl phenol in 89% yield (Scheme 2).

Considering this excellent result, we looked for an effective procedure for the preparation of 2,5-dihydroxy-3,4-dimethylbenzoic acid **5**, which should then be subsequently reduced applying the same protocol, affording the deuterated hydroquinone **11** (Scheme 3).

For the introduction of a carboxylic group into 2,3-dimethylhydroquinone (DMHQ) we planned to use a metalation reaction. For this purpose, as the use of $CO_2$ as electrophile gave unsatisfying results both by direct and bromine−lithium exchange metalation, we prepared the ethyl ester **4**.[12] Subsequent saponification and deprotection provided the desired acid **5** in 86% yield. High selectivity for ring over benzylic metalation was achieved using *t*-BuLi as metalating agent in hexane at 0 °C.[13] Hence, carbonate **6** was obtained in 70% yield from **5** by applying the reduction protocol mentioned above.[11,14]

However, removing the ethyl formate group to obtain **7** was tougher than expected. Applying strong acidic conditions (TFA and HCl) in a $H_2O$/THF mixture to **6**, as well as mild saponification by LiOH in MeOH, gave poor results (25% and 55%, respectively). Heating the carbonate at 50 °C in methanolic $H_2SO_4$ (10% v/v) gave slightly better but still unsatisfactory results (62% at best).

To avoid carbonate formation, we tried to selectively remove the MOM group of **4** ortho to the ester moiety, thus leaving the other phenolic moiety protected by the MOM group during the reduction step. Substrates containing two MOM groups can be differentiated if one is ortho to a chelating group, like a carbonyl group. Such selective deprotection has been achieved on several substrates using montmorillonite K 10[15] and the $I_2$/MeOH

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system.[16] However, we decided to follow the procedure of Haraldsson regarding the selective cleavage of aromatic benzyl ethers ortho to a carbonyl group with $MgBr_2$.[17] Stirring a mixture of $MgBr_2$ and **4** in benzene overnight at room temperature proved indeed to be very effective, allowing the recovery of desired **8** in 91% yield. Saponification afforded acid **9** quantitatively. Much to our delight, treatment of **9** with ethyl chloroformate, followed by reduction of the corresponding bis-ethoxycarbonyl derivative with excess $NaBD_4$ in $D_2O$/THF solution and *p*-TsOH-catalyzed deprotection, delivered **11** in an overall yield of 80% from **8**. Condensation of **11** with 1 in the presence of $BF_3{\cdot}Et_2O$[8] eventually afforded the desired α-CEHC-$d_3$ **12** in 79% yield with an excellent 97.5% isotopic purity. Moreover, α-tocopheronolactone-$d_3$ **13** was easily prepared by $FeCl_3{\cdot}6H_2O$ oxidation without any loss of the level of deuteration, as confirmed by GC−MS analysis (Scheme 3).

In summary, the first synthesis of α-CEHC-$d_3$ has been developed through an efficient way for the preparation of the key building block **11**, in 57% yield over seven steps and 97.5% isotope purity. In particular, the mild selective deprotection of **4** by $MgBr_2$ and the two-step reduction of salicylic acid **9** to the corresponding 2-$CD_3$-phenol **10** resulted quite effective and promising for further development. Results from a study evaluating the scope of this unusually easy reduction sequence will be reported in due course. Labeled hydroquinone **11** can be also used in the preparation of other labeled tocopherol derivatives and metabolites.

## Experimental Section

**General Methods.** All reactions were performed under inert atmosphere (argon or $N_2$). All melting points are uncorrected. NMR spectra were obtained at 200 MHz ($^1$H), 50.3 MHz ($^{13}$C), and 46 MHz ($^2$H) using $Me_4Si$, $CHCl_3$, or $CDCl_3$ as internal standards. Chemical shifts are expressed on the δ scale (ppm). Deuteration level was determined by GC−MS by microSIS mode, monitoring from *m/z* 422 to *m/z* 428 and from *m/z* 276 to *m/z* 282 for **12** (TMS derivative) and **13**, respectively, using a DB-5 capillary column (30 m × 0.25 mm, 0.25 μm film thickness). Operating conditions: injector temperature 280 °C; oven program temperature 250 °C, increased at 30°/min to 300 °C, and held for 7 min at 300 °C; transfer line temperature 295 °C; ion trap temperature 250 °C; emission current 10 μA; isolation window 3 amu. Hexane and 1,4-dioxane were distilled over $CaH_2$ and Na, respectively, before use. DMF employed was HPLC grade, kept over molecular sieves (4 Å). $NaBD_4$ (98 atom % D) and $D_2O$ (99.8 atom % D) were purchased from Aldrich. All other commercial reagents were used without further purification. Column chromatography was performed on silica gel 60 (70−230 mesh). TLC was performed on silica gel Macherey-Nagel Alugram Sil G/$UV_{254}$ (0.20 mm). Workup involved addition of water and 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.

**1,4-Bis(methoxymethoxy)-2,3-dimethylbenzene (3).** To a suspension of dry NaH (6.2 g, 260 mmol, 2.6 equiv) in DMF (40 mL) was added dropwise a solution of 2,3-dimethylhydroquinone (13.8 g, 100 mmol) in DMF (50 mL) at 0 °C. The mixture was allowed to warm to room temperature and stirred for 1 h. After cooling again to 0 °C, a solution of MOMCl (19.7 mL, 260 mmol, 2.6 equiv) in DMF (20 mL) was added dropwise, and the resulting mixture was stirred overnight at rt. Complete conversion of the starting phenol was confirmed by TLC (Hex/EtOAc 6:1). After workup in $Et_2O$, the residue obtained was filtered through a plug of silica gel (Hex/EtOAc 6:1) to give **3** (21.02 g, yield 93%) as pale yellow oil. $^1$H NMR ($CDCl_3$/TMS): δ 2.21 (s, 6 H), 3.52 (s, 6 H), 5.10 (s, 4 H), 6.92 (s, 2 H). $^{13}$C NMR ($CDCl_3$): δ 12.3, 55.9, 95.4, 112.5, 127.6, 150.3.

**Ethyl 2,5-Bis(methoxymethoxy)-3,4-dimethylbenzoate (4).** to a solution of **3** (15.7 g, 69.5 mmol) in dry hexane (100 mL) was added dropwise a solution of *t*-BuLi (45 mL, 76.4 mmol, 1.1 equiv) in pentane at 0 °C, and the resulting mixture was stirred for 3 h at 0 °C. Then dry hexane (40 mL) and $ClCO_2Et$ (60 mL, 625.5 mmol, 9 equiv) were added in one portion, and the reaction was stirred at 0 °C for an additional 3 h. Then the reaction was quenched with 1 M NaOH (40 mL). After workup in $Et_2O$, the residue obtained was purified by column chromatography (Hex/EtOAc 8:1), affording **4** (17.4 g, 84% yield) as a yellow semisolid liquid. $^1$H NMR ($CDCl_3$/TMS): δ 1.35 (t, $J$ = 6.8 Hz, 3 H), 2.18 (s, 3 H), 2.24 (s, 3 H), 3.45 (s, 3 H), 3.55 (s, 3 H), 4.31 (q, $J$ = 6.8 Hz, 2 H), 4.95 (s, 2 H), 5.14 (s, 2 H), 7.33 (s,1 H). $^{13}$C NMR ($CDCl_3$): δ 12.8, 13.3, 14.2, 56.0, 57.5, 60.8, 95.0, 101.2, 113.8, 122.2, 132.5, 132.8, 150.4, 151.0, 166.1.

**2,5-Dihydroxy-3,4-dimethylbenzoic Acid (5).** To a solution of **4** (2.7 g, 9 mmol) in THF (15 mL) was added a solution of KOH 4 M (10 mL) in $H_2O$/EtOH 1:1, and the mixture was stirred overnight. Complete saponification of starting **4** was confirmed by TLC (Hex/EtOAc 6:100). EtOH was evaporated, and $HCl_{conc}$ was added until the mixture was made acid. After workup in $Et_2O$, the residue was dissolved in a THF/$H_2O$/$HCl_{conc}$ 12:4:2 (18 mL) mixture and heated at 50 °C for 2 h to complete the deprotection sequence. Workup in $Et_2O$ after THF evaporation afforded a residue that was filtered through a plug of silica gel (Hex/EtOAc/AcOH 5:1:0.01) to give **5** (1.43 g, yield 86%) as white-pale yellow solid. Mp: 208−211 °C. $^1$H NMR ($CDCl_3$ + $CD_3OD$/TMS): δ 2.07 (s, 3 H), 2.11 (s, 3 H), 5.81 (bs, 2 H), 7.09 (s,1 H), 10.62 (bs, 1 H). $^{13}$C NMR ($CDCl_3$ + $CD_3OD$/): δ 11.4, 12.6, 108.0, 111.3, 127.5, 133.5, 146.3, 156.3, 167.2.

**Ethyl 2-Hydroxy-5-methoxymethoxy-3,4-dimethylbenzoate (8).** To a suspension of $MgBr_2$ (1.03 g, 5.6 mmol) in benzene (25 mL) was added a solution of **4** (1.2 g, 4 mmol) in benzene (10 mL), and the mixture was stirred overnight, the suspension color changing from white to yellow. After addition of a saturated aqueous $NH_4Cl$ solution (10 mL), the mixture was stirred for 30 min. After workup in EtOAc, the crude mixture was purified by column chromatography (Hex/EtOAc 4:1), affording **8** (930 mg, 91% yield) as pale yellow solid. Mp: 64−66 °C. $^1$H NMR ($CDCl_3$/TMS): δ 1.39 (t, $J$ = 7.4 Hz, 3 H), 2.17 (s, 3 H), 2.20 (s, 3 H), 3.48 (s, 3 H), 4.36 (q, $J$ = 7.4 Hz, 2 H), 5.12 (s, 2 H), 7.32 (s, 1 H), 10.91 (s, 1 H). $^{13}$C NMR ($CDCl_3$): δ 11.2, 12.5, 13.8, 55.6, 60.7, 95.0, 108.3, 111.0, 125.6, 135.4, 147.0, 154.9, 170.0.

**2-Hydroxy-5-methoxymethoxy-3,4-dimethylbenzoic Acid (9).** Compound **8** (920 mg, 3.62 mmol) was dissolved in a methanolic solution of KOH (13 mL, 2M). The resulting solution was heated at 50 °C for 2.5 h, and then MeOH was evaporated and the pH adjusted to 4 with 1 M HCl. Workup in EtOAc gave pure **9** (820 mg, ∼100% yield), without further purification, as a white-pale yellow solid. Mp: 142−145 °C. $^1$H NMR ($CDCl_3$/TMS): δ 2.14 (s, 3 H), 2.18 (s, 3 H), 3.47 (s, 3 H), 5.10 (s, 2 H), 5.76 (bs, 2 H), 7.38 (s, 1 H). $^{13}$C NMR ($CDCl_3$): δ 12.6, 13.9, 57.1, 96.8, 111.9, 114.0, 126.7, 136.2, 148.6, 156.4, 175.1.

**4-Methoxymethoxy-(2-$^2H_3$)-5,6-trimethylphenol (10).** To a solution of **9** (800 mg, 3.54 mmol) and $Et_3N$ (1.2 mL, 8.6 mmol, 2.4 equiv) in THF (50 mL) at 0 °C was added ethyl chloroformate (0.82 mL, 8.6 mmol, 2.4 equiv), and the mixture was stirred for 3 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 $NaBD_4$ (1.19 g, 28.5 mmol, 8 equiv) in $D_2O$ (10 mL) and THF (5 mL) at 0 °C. After 3 h at 0 °C, the white suspension was allowed to rise to rt and stirred overnight.

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TLC (Hex/EtOAc/AcOH 4:1:0.005) showed complete conversion of the bis-carbonate intermediate. The pH of the mixture was then adjusted to 6 with 1 M HCl and AcOH and THF evaporated. After workup in $Et_2O$, the residue was purified by column chromatography (Hex/EtOAc 4:1), affording **10** (630 mg, 89% yield) as a white solid. Mp: 92−93 °C. $^1$H NMR ($CDCl_3$/TMS): $\delta$ 2.18 (s, 6 H), 3.53 (s, 2 H), 5.03 (s, 1 H), 5.11 (s, 2 H), 6.73 (s,1 H). $^2$H NMR ($CHCl_3$): $\delta$ 2.19 (s). $^{13}$C NMR ($CDCl_3$): $\delta$ 12.1, 13.7 (m), 55.8, 95.7, 115.1, 120.7, 123.7, 124.9, 147.1, 148.6. APCI-MS (in MeOH), *m/z* (amu): negative ion mode, 198 ($M - H^+$).

**(2-$^2H_3$)-5,6-Trimethylhydroquinone (11).** A solution of **10** (590 mg, 2.96 mmol) and catalytic *p*-TsOH in MeOH (25 mL) was heated at 50 °C for 3 h. TLC (Hex/EtOAc 6:1) confirmed complete conversion of **10**. After evaporation of the solvent, the residue was purified by column chromatography (Hex/EtOAc 6:1), affording **11** (480 mg, 90% yield) as a white solid. Mp: 178−181 °C. $^1$H NMR ($CD_3OD$/TMS): $\delta$ 2.02 (s, 3 H), 2.06 (s, 3 H), 3.77 (s, 2 H), 6.35 (s,1 H). $^2$H NMR ($CH_3OH$): $\delta$ 2.44 (s). $^{13}$C NMR ($CD_3OD$): $\delta$ 11.6, 12.1, 13.6 (m), 113.9, 121.1, 121.8, 124.5, 146.3, 147.5. APCI-MS (in MeOH), *m/z* (amu): negative ion mode, 154 ($M - H)^-$. Anal. Calcd for $C_9H_9D_3O_2$ (155.2): C, 69.65; H, 9.74. Found: C, 69.43; H, 9.93; 97.5% isotope purity by GC−MS spectrometry.

**(5-$^2H_3$)-2,7,8-Tetramethyl-2-(2′-carboxyethyl)-6-hydroxychroman (α-CEHC-*d*$_3$) (12).** A dry dioxane (10 mL) solution of lactone **2** (1.22 g, 9.63 mmol), prepared following the procedure reported by Wechter et al.,[9] was added over 4 h to a solution of **11** (990 mg, 6.42 mmol) and $BF_3{\cdot}Et_2O$ (0.6 mL, 5 mmol) in dry dioxane (8 mL) at 110 °C while stirring. After an additional 1 h at 110 °C, the reaction mixture was cooled to room temperature, the solvent was evaporated, and the residue was dissolved in EtOAc. After workup in EtOAc, the crude brownish product obtained was purified by column chromatography (Hex/EtOAc/AcOH 3:5:0.01) to give **12** as a beige powder (1.44 g, 79%). Mp: 176−178 °C. $^1$H NMR ($CD_3OD$/TMS): $\delta$ 1.18 (s, 3 H), 1.72−1.93 (m, 4 H), 2.04 (s, 3 H), 2.11 (s, 3 H), 2.39−2.47 (m, 2 H), 2.57 (t, $J$ = 4.4 Hz, 2 H), 4.96 (s, 2 H). $^2$H NMR ($CH_3OH$): $\delta$ 2.14 (s). $^{13}$C NMR ($CD_3OD$/TMS): $\delta$ 11.2 (m), 12.1, 12.9, 21.6, 23.7, 29.5, 32.7, 35.5, 74.5, 118.0, 121.8, 123.1, 124.4, 146.1, 146.3, 177.7. APCI-MS (in MeOH), *m/z* (amu): positive ion mode, 282 $(M + H)^+$, 299 $(M + NH_4)^+$, 304 $(M + Na)^+$. Anal. Calcd for $C_{16}H_{19}D_3O_4$ (281.2): C, 68.30; H, 8.95. Found: C, 68.20; H, 8.87; 97.5% isotope purity by GC−MS (TMS derivative).

**4-Methyl-6-[(3$^2H_3$)-5,6-trimethylbenzochinoyl]-4-hexanolide (13).** Oxidation of **12** (500 mg, 1.78 mmol) by solution of $FeCl_3{\cdot}6H_2O$ in MeOH/$H_2O$ 1:1, following the procedure reported by Kantoci et al.,[8] afforded **13** (420 mg, 82% yield) as yellow solid. Mp: 66−68 °C. $^1$H NMR, ($CDCl_3$/TMS): $\delta$ 1.39 (s, 3 H), 1.62−1.69 (m, 2 H), 1.92 (s, 6 H), 1.96−2.28 (m, 2 H), 2.46−2.67 (m, 4 H). $^2$H NMR ($CH_3OH$): $\delta$ 1.95 (s, $ArCD_3$). $^{13}$C NMR ($CD_3OD$/TMS): $\delta$ 11.9 (m), 12.1, 12.2, 21.1, 25.3, 28.8, 32.4, 39.1, 85.9, 140.2, 140.5, 142.8, 153.4, 176.2, 186.8, 187.2. APCI-MS (in MeOH), *m/z* (amu): positive ion mode, 280 $(M + H)^+$, 297 $(M + NH_4)^+$, 302 $(M + Na)^+$. Anal. Calcd for $C_{16}H_{17}D_3O_4$ (279.2): C, 68.79; H, 8.30. Found: C, 68.59; H, 8.15; 97.5% isotope purity by GC−MS.

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