Synthesis and Spectroscopic Characterization of [1'-¹⁴C]Ubiquinone-2, [1'-¹⁴C]-5-Demethoxy-5-hydroxyubiquinone-2, and [1'-¹⁴C]-5-Demethoxyubiquinone-2

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[1'-¹⁴C]Ubiquinone-2, [1'-¹⁴C]-5-demethoxy-5-hydroxyu-biquinone-2, and [1'-¹⁴C]-5-demethoxyubiquinone-2 have been synthesised from [1-¹⁴C]acetic acid. A common feature of these benzoquinones is the isoprenoid chain, and the ¹⁴C-label has therefore been incorporated in this isoprenoid. The coupling of the different quinone head groups and the isoprenoid chain is the last step in the total synthesis, to prevent unnecessary loss of the labelled material during synthesis. The products have been characterised by mass spectrometry, ¹H NMR and ¹³C NMR.

Introduction

Quinones are found in biomembranes, where they function as electron carriers and scavengers of free radicals.[1,2] Several types of quinones are known to function as essential electron transporters in the electron-transport chain of many organisms.

Ubiquinone, a 1,4-benzoquinone, functions in bacteria and eukaryotes as an electron transporter in aerobic metabolism.[1,2] Deficiencies in the amounts of quinones are known to result in malfunctioning of the organism, and supplementation with quinones is used in the treatment of patients with cardiovascular diseases.[3]

In contrast to this aerobically functioning electron transporter, an anaerobically functioning electron transporter, rhodoquinone, is found in several (facultative) anaerobically functioning eukaryotes, such as parasitic helminths, sea mussels and oysters.[4] As parasitic diseases are still a serious threat to the health of humans and animals,[5,6] new antiparasitic drugs are urgently needed. When parasitic helminths have invaded the host animal, the metabolism of the parasite often switches from aerobic to anaerobic, and then uses rhodoquinone as an electron transporter.[4,7] As rhodoquinone is absent in their hosts, the biosynthesis of rhodoquinone could be a suitable target for antiparasitic drugs and deserves further investigation.

The biosynthesis of ubiquinone is completely known.[8] The only difference between rhodoquinone and ubiquinone
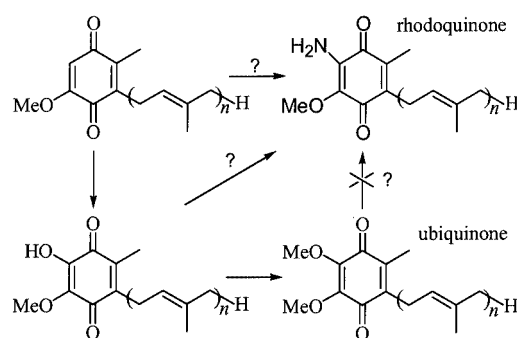

Figure 1. The last three steps of the ubiquinone biosynthesis in eukaryotic organisms, and the proposed precursors of rhodoquinone biosynthesis; in mammals and rats, $n = 10$ and $n = 9$, respectively; in this paper systems with $n = 2$ have been prepared

is an amino group in place of a methoxy group at the 5-position (Figure 1). The biosynthesis of rhodoquinone is believed to be very similar to that of ubiquinone, except for the last step (Figure 1). To unravel the biosynthetic route to rhodoquinone, ¹⁴C-labelled ubiquinone biosynthesis intermediates are needed. We have therefore optimised synthetic schemes for the incorporation of a ¹⁴C-label in the 1'-position of the side chain in the following compounds: ubiquinone-2 [5,6-dimethoxy-3-methyl-2-diprenylbenzoquinone, see Figure 2; diprenyl = 3,7-dimethyl-2,6-octadienoyl (= geranyl)], 5-demethoxy-5-hydroxyubiquinone-2 (5-hydroxy-6-methoxy-3-methyl-2-diprenylbenzoquinone, see Figure 2), and 5-demethoxyubiquinone-2 (6-methoxy-3-methyl-2-diprenylbenzoquinone, see Figure 2). The synthesis of these quinones will be described in this article.

Results and Discussion

To enable the introduction of the ¹⁴C-label, the synthesis of the isoprenoid chain started from acetic acid (Scheme 1).

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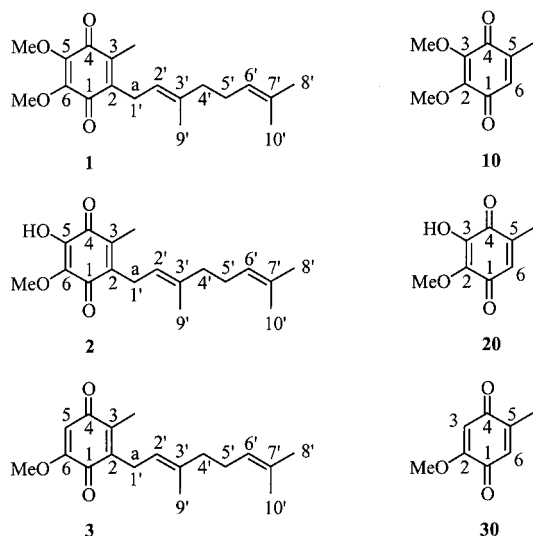E-mail: lugtenbu@chem.leidenuniv.nl

Figure 2. Structure and IUPAC numbering of [1′-^{12}C]ubiquinone-2 (**1**), [1′-^{14}C]ubiquinone-2 (**1a**), [1′-^{12}C]-5-demethoxy-5-hydroxyubiquinone-2 (**2**), [1′-^{14}C]-5-demethoxyubiquinone-2 (**2a**), [1′-^{12}C]-5-demethoxyubiquinone-2 (**3**), [1′-^{14}C]-5-demethoxyubiquinone-2 (**3a**), ubiquinone-0 (**10**), 3-demethoxy-3-hydroxyubiquinone-0 (**20**), and 3-demethoxyubiquinone-0 (**30**) ("a" indicates the position of the ^{14}C-label); note the difference in the numbering between the quinones with and the quinones without isoprenoid chains

Tri-*n*-butyl(geranyl)tin (**13**) was used for the synthesis of ubiquinone-2 (**1**) and 5-demethoxy-5-hydroxyubiquinone-2 (**2**), while geranyl bromide (**14**) was used for the synthesis of 5-demethoxyubiquinone-2 (**3**). The coupling of the different quinone head groups and the isoprenoid chains is the last step in the total synthesis, so as to prevent unnecessary loss of the radioactive material during synthesis. For this reason we developed novel and more efficient synthetic schemes for these compounds. These schemes were first optimised using only unlabelled starting material.

Synthesis of Geranyl Bromide (14)

Acetic acid (**17**) was selected as starting material, as we wanted to insert a ^{14}C-label into the isoprenoid chain and as [1-^{14}C]sodium acetate in water is commercially available. The water was removed by co-evaporation with toluene, and the sodium acetate was subsequently dissolved in an excess of unlabelled acetic acid, as the total amount was otherwise too small to perform the synthesis with. It was not possible to use more of the undiluted labelled material, as the permitted total amount of radioactivity is limited.
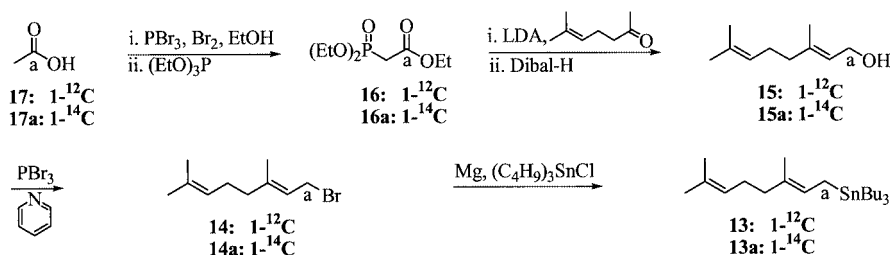
The synthesis of ethyl bromoacetate was performed by a Hell−Volhard−Zelinsky approach, acetic acid (**17**) being brominated with freshly distilled PBr_3 and Br_2 and subsequently esterified with freshly distilled absolute ethanol.[9] Purification by distillation gave ethyl bromoacetate in quantitative yield (Scheme 1). Triethyl phosphite was added to ethyl bromoacetate, to yield triethyl phosphonoacetate (**16**) by an Arbusov reaction in quantitative yield after distillation under reduced pressure. 6-Methyl-5-hepten-2-one was treated with lithiated triethyl phosphonoacetate in a Wittig reaction to yield pure ethyl 3,7-dimethyl-2,6-octadienoate (81%) after distillation. This metallation of triethyl phosphonoacetate (**16**) with NaH has also been described,[10] but we preferred *n*BuLi for the metallation, as this reagent reacts faster and is easier to work with. Furthermore the product ethyl 3,7-dimethyl-2,6-octadienoate was obtained in an apparently higher yield. This compound was reduced to geraniol (**15**) with Dibal-H in 92% yield after vacuum distillation.[10] Geraniol (**15**) was brominated with freshly distilled PBr_3 in the presence of a catalytic amount of pyridine[10] in 97% yield. In earlier attempts the bromination of geraniol (**15**) gave more then 100% yield, probably caused by the presence of HBr in the product due to the use of impure PBr_3. Therefore the use of very pure or distilled PBr_3 is recommended. Geranyl bromide was purified by vacuum distillation at 67−70 °C. This compound should not be heated above 80 °C, because of the thermal lability of geranyl bromide (**14**).

The same route was used for the synthesis of [1-^{14}C]geranyl bromide (**14a**).

## Synthesis of Tri-*n*-butyl(geranyl)tin (13)

Tri-*n*-butyl(geranyl)tin (**13**) has been synthesised by a Barbier cross−coupling of geranyl bromide (**14**) and tri-*n*-butyltin chloride in the presence of activated magnesium[11] (Scheme 1). Vacuum distillation gave the pure product in 65% yield. This method proved to be more reliable than a method described earlier,[12] in which lithium diisopropylamide and tri-*n*-butyltin hydride were used as reagents. The same route was used for the synthesis of tri-*n*-butyl([1-^{14}C]geranyl)tin (**13a**).

Synthesis of 3-Demethoxy-3-hydroxyubiquinone-0 (20)

With respect to the synthesis of 5-demethoxy-5-hydroxyubiquinone-2, it might seem a quick approach to remove the methyl group from the 5-methoxy group of ubiquinone-

Scheme 1. Synthesis of geranyl bromide (**14**) and tri-*n*-butyl(geranyl)tin (**13**) ("a" indicates the position of the ^{14}C-label)

2. However, this approach is not specific, as there are two methoxy groups present, and so this should result in an isomeric mixture of 5-demethoxy-5-hydroxyubiquinone-2 and 6-demethoxy-6-hydroxyubiquinone-2. A novel synthetic scheme for the synthesis of 5-demethoxy-5-hydroxyubiquinone-2 was therefore designed.

For the synthesis of 2,3-dihydroxy-4,5-dimethoxytoluene (**6**) from 3,4,5-trimethoxytoluene (**7**; Scheme 2) a 2modification of the procedure described by Baker and Raistrick[13] was used. Friedel−Crafts acylation of 3,4,5-trimethoxytoluene (**7**) was performed by heating the reaction mixture under reflux with excess of $AlCl_3$ and acetyl chloride for 3 h. Pure 2-acetyl-3-hydroxy-4,5-dimethoxytoluene was obtained by recrystallization, in 64% yield. The acetyl group was substituted by a hydroxy group by means of a modified Dakin reaction with NaOH and H_2O_2. Purification of the crude compound by recrystallization yielded 95% of 2,3-dihydroxy-4,5-dimethoxytoluene (**6**). Oxidation of this with sodium chlorate in the presence of a catalytic amount of V_2O_5[14] gave the corresponding ubiquinone derivative **20** in 95% yield. A previously described CAN oxidation[15] on compound **6** was not reproducible in our hands.
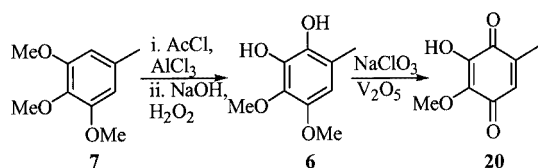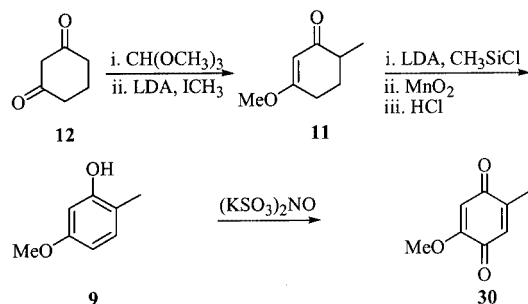

Scheme 2. Synthesis of 3-demethoxy-3-hydroxy-ubiquinone-0 (**20**)

Synthesis of 3-Demethoxyubiquinone-0 (30)

To convert 1,3-cyclohexadione (**12**) into 3-methoxy-6-methylphenol (**9**), in four steps, an optimised synthesis adapted from Boers was performed.[12]

Treatment of 1,3-cyclohexadione (**12**) with trimethyl orthoformate in the presence of acid and under nitrogen gave 3-methoxy-2-cyclohexenone in 95% yield (Scheme 3). This reaction was a modification of the literature procedure.[16−18] 3-Methoxy-2-cyclohexenone was metallated at the 6-position with lithium diisopropylamide and subsequently methylated with iodomethane, yielding 3-methoxy-6-methyl-2-cyclohexenone (**11**, 88%). After lithiation of this with lithium diisopropylamide and silyl-

Scheme 3. Synthesis of 3-demethoxyubiquinone-0 (**30**)

ation with chlorotrimethylsilane, the corresponding trimethylsilyl ether was obtained in 97% yield. This was oxidized to 3-methoxy-6-methyl-1-(trimethylsiloxy)benzene with MnO_2 in 96% yield and subsequently treated with acid to remove the trimethylsilyl group (90% yield). Purification by distillation afforded the phenol **9** in a total yield of 70% from 1,3-cyclohexadione (**12**).

Oxidation of this phenol derivative **9** with potassium nitrosodisulfonate (Fremy's salt)[19,20] gave ubiquinone-0 (**30**), which was purified by silica gel chromatography to yield the benzoquinone **30** (68%).

Synthesis of Ubiquinone-2 (1)

This synthesis was performed in one step from commercially available ubiquinone-0 (**10**) (Scheme 4). Tri-*n*-butyl-(geranyl)tin (**13**) was coupled to the ring structure in 90% yield with boron trifluoride−diethyl ether as catalyst and $FeCl_3$ as oxidant, as described in the literature.[21,22] The same route was used for the synthesis of [1′-¹⁴C]ubiquinone-2 (**1a**).
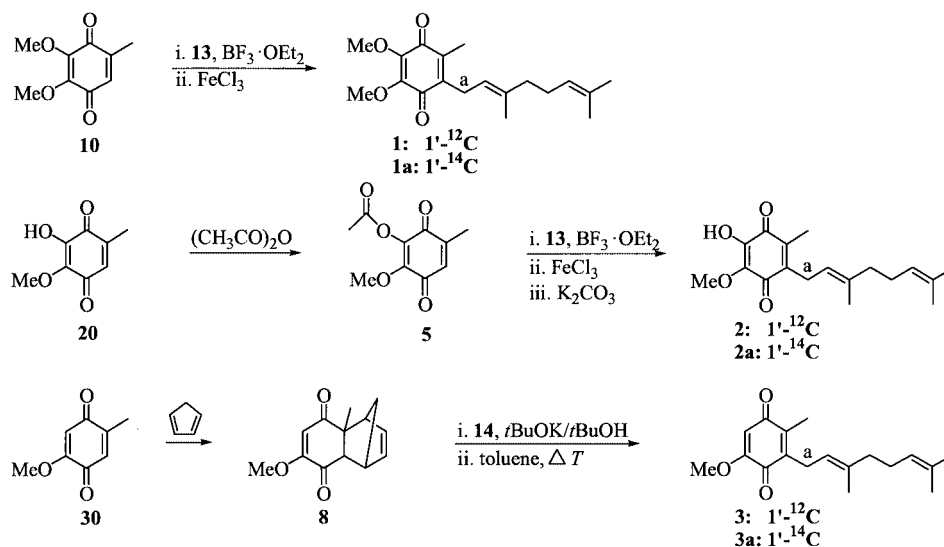
Synthesis of 5-Hydroxy-5-demethoxyubiquinone-2 (2)

No coupling reaction between 3-demethoxy-3-hydroxyubiquinone-0 (**20**) and tri-*n*-butyl(geranyl)tin (**13**) was observed. We therefore protected the hydroxy group with an acetyl group, by heating under reflux in acetic anhydride (Scheme 4). Tri-*n*-butyl(geranyl)tin (**13**) was coupled, by a kind of Sakurai reaction,[23,24] to 3-acetoxy-2-methoxy-5-methyl-1,4-benzoquinone (**5**). It is important to keep the temperature of this reaction low (about −60 °C), as higher temperatures result in a variety of undefined side-products. This is in contrast to the coupling of tri-*n*-butyl(geranyl)tin (**13**) to ubiquinone-0 (**10**), which was performed from −30 °C to room temperature. After purification on silica gel, 3-acetoxy-6-geranyl-2-methoxy-4-methyl-*p*-benzoquinone was obtained in 75% yield. It is important to perform the silica gel purification at this point, as it is not possible to purify the end product **2** on silica gel, due to its instability. The *O*-acetyl group was removed by addition of a dilute K_2CO_3 solution. As the presence of oxygen in water destroys the product, this reaction should be performed under nitrogen. We therefore passed nitrogen gas through the reaction mixture during the whole procedure. The product **2** was purified by an extraction procedure with base and acid under exclusion of air and the very pure product was obtained in 84% yield.

The same route was used for the synthesis of [1′-¹⁴C]-5-demethoxy-5-hydroxyubiquinone-2 (**2a**).

Synthesis of 5-Demethoxyubiquinone-2 (3)

To couple geranyl bromide (**14**) to 3-demethoxyubiquinone-0 (**30**), the latter compound was protected by performing a cycloaddition reaction with cyclopentadiene, the Diels−Alder adduct **8** being obtained in 87% yield (Scheme 4). Treatment of **8** with geranyl bromide (**14**) in the presence of *t*BuOK yielded 56% of the geranyl-substituted derivative. As the metallated compound **8** is not stable, both

Scheme 4. Synthesis of ubiquinone-2 (**1**), 5-demethoxy-5-hydroxyubiquinone-2 (**2**), and 5-demethoxyubiquinone-2 (**3**), from ubiquinone-0 (**10**; commercially available), 3-demethoxy-3-hydroxyubiquinone-0 (**20**; from Scheme 2) and 3-demethoxyubiquinone-0 (**30**; from Scheme 3), respectively ("a" indicates the position of the ^{14}C-label; **13** and **14** from Scheme 1)

reagents (**8** and **14**) were added simultaneously at 0 °C to an excess of the *tert*-butoxide. After purification on silica gel, the geranyl derivative was heated under reflux in toluene (to remove the cyclopentadiene group), and 5-demethoxyubiquinone-2 (**3**) was obtained in quantitative yield.

The same route was used for the synthesis of [1′-^{14}C]-5-demethoxyubiquinone-2 (**3a**).

Analysis of the End Products

All the end products had a purity of at least 97% and were characterised by TLC analysis, mass spectrometry, and ^{1}H and ^{13}C NMR spectroscopy.

Our procedure ensured that the label was present only in the isoprenoid side chain, as the synthesis started with acetic acid as the only labelled compound. The unlabelled and the labelled quinones were compared by TLC analysis. The colours and reference values agreed for both products. After isolation of the spots corresponding to the [1′-^{14}C]ubiquinones from the TLC plates, the specific activities of the compounds were determined. They were in agreement with the specific activities of acetic acid at the start of the synthesis.

The exact masses of **1**, **2**, **3**, **20**, and **30** were determined, and these masses were the same (within experimental error) as their calculated values.

The ^{1}H NMR spectrum of ubiquinone-2 (**1**) was compared to the spectrum of commercially available ubiquinone-10.[16] The only differences found for **1** were due to the shorter length of the isoprenoid chain. The ^{1}H NMR spectra of 5-demethoxy-5-hydroxyubiquinone-2 (**2**) and 5-demethoxyubiquinone-2 (**3**) were compared to the spectrum of **1**. For **2**, one of the methoxy substituents ($\delta = 3.99$ ppm) was replaced by an extra, broadened signal at $\delta = 6.50$ ppm (OH), while for **3** this methoxy substituent was replaced by an extra singlet at $\delta = 5.88$ ppm (H).

Experimental Section

General: All reactions were performed under dry nitrogen, unless stated otherwise, and dry organic solvents, purified and dried by standard methods, were used. The reaction temperatures given are internal temperatures. All chemicals were obtained from Aldrich or Acros Chimica. NMR spectra were measured with Bruker WM 300 or Bruker AM 600 spectrometers. Proton spectra were measured in deuteriochloroform with tetramethylsilane as an internal standard ($\delta = 0.0$ ppm), and carbon-13 spectra were measured in deuteriochloroform as an internal standard ($\delta = 77$ ppm). Mass spectra were performed with a Finnigan MAT 900 (equipped with a direct insertion probe), with a Finnigan MAT 900 (equipped with an electrospray interface), or with a Jeol AX 505 W (equipped with a direct insertion probe). Melting points were determined with a Büchi melting point apparatus and are uncorrected. Refraction indices were measured with a Jena refractometer. TLC analysis was performed on Merck 60 F254 silica gel plates and spots were viewed under UV light or by staining with phosphomolybdic acid in ethanol or with ammonium molybdate tetrahydrate and ammonium cerium(IV) sulfate dihydrate in a 10% sulfuric acid solution. Purification was performed by flame distillation or by silica gel chromatography. The specific activities of the ^{14}C-labelled end products were determined with a Packard 2200CA TRI-CARB Liquid Scintillation Analyser.

Synthesis of Geranyl Bromide (14)

[1-^{14}C]Acetic Acid (17a): [1-^{14}C]Sodium acetate (74 MBq) in water (5 mL) with a specific activity of 2.10^3 MBq/mmol was purchased. Toluene was added to remove water by evaporation in vacuo. The [1-^{14}C]sodium acetate was dissolved in acetic acid (1.0 g, 16.7 mmol). The specific activity of this solution was determined to be 3.7 MBq/mmol. This specific activity did not change during the synthesis.

Ethyl [1-^{14}C]Bromoacetate and Ethyl Bromoacetate: Phosphorus tribromide (4.64 g, 17.1 mmol) was added to acetic acid (**17**, 1.00 g, 16.6 mmol) under nitrogen. Bromine (6.08 g, 38.1 mmol) was added over 3 min through a cooler, as this caused an exothermic reaction. After completion of the addition the reaction mixture was red

and it was heated under reflux for 75 min during which a large quantity of HBr was evolved, resulting in a clear orange reaction mixture. Freshly distilled absolute ethanol (2.0 g, 43 mmol) was added at room temp. over 3 min, whereupon the temperature rose to 80 °C. After cooling to room temp., the reaction mixture was heated under reflux for 30 min with evolution of HBr, after which the reaction mixture still had a reddish colour. The product was purified by flame distillation at atmospheric pressure under a nitrogen stream to remove the last traces of dissolved HBr. Ethyl bromoacetate was obtained at 155 °C as a colourless liquid in a quantitative yield (2.80 g, 16.6 mmol). ¹H NMR (CDCl₃, 300 MHz): δ = 4.24 (q, $^3J_{H-H}$ = 7.1 Hz, 2 H, OCH₂), 3.84 (s, 2 H, CH₂), 1.31 (t, $^3J_{H-H}$ = 7.1 Hz, 3 H, CH₃) ppm. ¹³C NMR (CDCl₃, 75 MHz): δ = 62.3 (CH₂), 26.3 (CH₂), 14.3 (CH₃) ppm. MS (ESI): m/z = 167.

Triethyl [1-¹⁴C]Phosphonoacetate (16a) and Triethyl Phosphonoacetate (16): Triethyl phosphite (3.4 g, 20 mmol) was added to ethyl bromoacetate (2.80 g, 16.6 mmol). The reaction mixture was heated under reflux at 60−80 °C for 30 min, during which some ethyl bromide evolved. After the mixture had cooled to room temp., ethyl bromide was distilled off at atmospheric pressure (b.p. 37−40 °C). Triethyl phosphonoacetate (16) was purified by vacuum distillation at 20 mbar. After a small forerun, mainly consisting of the excess triethyl phosphite, triethyl phosphonoacetate (16) was collected at 144−146 °C. A colourless liquid was obtained in a quantitative yield (3.74 g, 16.6 mmol). ¹H NMR (CDCl₃, 300 MHz): δ = 4.17 (m, 6 H, 3 CH₂), 2.96 (d, $^2J_{H-H}$ = 21.5 Hz, 2 H, CH₂), 1.33 (m, 9 H, 3 CH₃) ppm. ¹³C NMR (CDCl₃, 75 MHz): δ = 165.6 (C=O), 62.4 (CH₂), 61.3 (CH₂), 35.0 (CH₂), 33.2 (CH₂), 16.2 (CH₃), 16.0 (CH₃), 13.7 (CH₃) ppm. MS (ESI): m/z = 225 [M + H], 247 [M + Na].

Ethyl [1-¹⁴C]3,7-Dimethyl-2,6-octadienoate and Ethyl 3,7-Dimethyl-2,6-octadienoate: Triethyl phosphonoacetate (16, 3.74 g, 16.7 mmol) in THF (20 mL) under nitrogen was metallated by addition by syringe, at −60 °C, of a slight excess of a BuLi solution in hexanes (1.6 M, 11.0 mL, 17.6 mmol), over 5 min. The temperature rose to −20 °C despite continuous cooling, and the reaction mixture was stirred for 20 min. A solution of 6-methyl-5-hepten-2-one (2.11 g, 16.7 mmol) in THF (5 mL) was added in one portion at −20 °C to the slightly yellow solution of the lithiated phosphonoacetate, and the reaction mixture was stirred for 20 h at room temp., after which it was orange-red. The reaction mixture was quenched in saturated aqueous ammonium chloride, the water phase was extracted with ether (3 × 20 mL) and dried with MgSO₄, and the solvents were evaporated in vacuo. The product was purified by vacuum distillation at 22 mbar at 120−122 °C. A light yellow liquid was obtained with a yield of 2.65 g (13.5 mmol, 81%). ¹H NMR (CDCl₃, 300 MHz): δ = 5.64 (s, 1 H, CH), 5.05 (t, $^3J_{H-H}$ = 1.4 Hz, 1 H, CH), 4.12 (q, $^3J_{H-H}$ = 7.1 Hz, 2 H, CH₂), 2.14 (s, 3 H, CH₃), 2.14 (m, 3 H, ¹/₂ CH₂ + CH₂) 1.86 (s, 1 H, ¹/₂ CH₂), 1.67 (s, 3 H, CH₃), 1.59 (s, 3 H, CH₃), 1.26 (t, $^3J_{H-H}$ = 7.1 Hz, 3 H, CH₃) ppm. ¹³C NMR (CDCl₃, 75 MHz): δ = 166.9 (C=O), 159.8 (C), 132.5 (C), 123.0 (CH), 115.5 (CH), 59.4 (CH₂), 40.9 (CH₂), 26.0 (CH₂), 25.6 (CH₃), 18.8 (CH₃), 17.7 (CH₃), 14.3 (CH₃) ppm. MS (ESI): m/z = 196.

[1-¹⁴C]-3,7-Dimethyl-2,6-octadienol (Geraniol, 15a) and Geraniol (15): Diisopropylbutylaluminium hydride in hexanes (1.0 M, 40 mL, 40 mmol) was added by syringe over 5 min, at −70 °C and under nitrogen, to a solution of ethyl 3,7-dimethyl-2,6-octadienoate (2.65 g, 13.5 mmol) in hexane (30 mL). The temperature of the reaction mixture immediately rose to −40 °C despite continuous cooling in liquid nitrogen and the reaction mixture turned yellow. When the addition was complete the cooling bath was removed

and the temperature rose to 10 °C in 10 min, whereupon the reaction mixture became colourless. By TLC spotting (20% ether in petroleum ether) it was concluded that the reaction was finished. After having been cooled to −80 °C, the reaction mixture was poured into a stirred mixture of silica gel and water (6.0 g of water in 15 g of silica gel). After the mixture had been stirred for 20 min at room temp., MgSO₄ was added for drying, the suspension was filtered through a glass filter, and the solid was washed thoroughly with ether. After removal of the solvents in vacuo, the residue was purified by vacuum distillation at 17 mbar at 108−110 °C; 15 was obtained as a colourless in a yield of 1.9 g (12.3 mmol, 92%). ¹H NMR (CDCl₃, 300 MHz): δ = 5.41 (t, $^3J_{H-H}$ = 6.9 Hz, 1 H, CH), 5.09 (m, 1 H, CH), 4.15 (d, $^3J_{H-H}$ = 6.9 Hz, 2 H, CH₂), 2.06 (m, 4 H, 2 CH₂), 1.68 (br. s, 6 H, 2 CH₃), 1.61 (s, 3 H, CH₃) ppm. ¹³C NMR (CDCl₃, 75 MHz): δ = 139.6 (C), 131.7 (C), 123.8 (CH), 123.3 (CH), 59.3 (CH₂), 39.5 (CH₂), 26.3 (CH₂), 25.6 (CH₃), 17.6 (CH₃), 16.2 (CH₃) ppm. MS (ESI): m/z = 154.

[1-¹⁴C]-3,7-Dimethyl-2,6-octadienyl Bromide (Geranyl Bromide, 14a) and Geranyl Bromide (14): Pyridine (0.08 g, 1.0 mmol) was added under nitrogen to a solution of geraniol (15, 1.9 g, 12 mmol) in ether (15 mL). A solution of freshly distilled PBr₃ (1.3 g, 4.8 mmol) in hexane (5 mL) was added over 15 min at 0 °C, during which a white suspension was initially formed and a slight exothermic effect of a few degrees was observed. The reaction mixture was stirred for 15 min, whereupon the solution had became clear and a pink precipitate of the phosphorous salts [P(OH)₃] had formed. TLC analysis (20% ether in petroleum ether) showed that all the geraniol had disappeared. After decantation of the yellow solution from the phosphorous salts into ice/water, the salts were washed with some hexane, which was also added to the ice/water. The layers were separated, the water phase was extracted with ether (3 × 20 mL), the combined organic fractions were washed extensively with brine (6 × 10 mL) and dried with MgSO₄, and the solvents were evaporated in vacuo. After vacuum distillation (b.p. 67−70 °C at 0.5 Torr), the pure geranyl bromide (14) was obtained as a colourless liquid in a yield of 2.55 g (11.7 mmol, 97%). n_D^{20} = 1.5022. ¹H NMR (CDCl₃, 300 MHz): δ = 5.53 (t, $^3J_{H-H}$ = 8.4 Hz, 1 H, CH), 5.07 (m, 1 H, CH), 4.02 (d, $^3J_{H-H}$ = 8.4 Hz, 2 H, CH₂), 2.09 (m, 4 H, 2 CH₂), 1.75 (s, 3 H, CH₃), 1.68 (s, 3 H, CH₃), 1.60 (s, 3 H, CH₃) ppm. ¹³C NMR (CDCl₃, 75 MHz): δ = 143.4 (C), 131.8 (C), 123.4 (CH), 120.5 (CH), 39.4 (CH₂), 25.8 (CH₃), 29.5 (CH₂), 26.1 (CH₂), 17.6 (CH₃), 15.8 (CH₃) ppm. MS (ESI): m/z = 217, 218 [M + H].

Synthesis of Tri-n-butyl(geranyl)tin (13)

Tri-n-butyl([1-¹⁴C]-3,7-dimethyl-2,6-octadienyl)tin {Tri-n-butyl([1-¹⁴C]geranyl)tin (13a)} and Tri-n-butyl(geranyl)tin (13): THF was added to magnesium turnings (0.60 g, 25 mmol) until all the magnesium was under the level of the solvent. The magnesium was activated by addition by pipette of a few drops of dibromoethane, until evolution of ethene was observed. As soon as the ethene evolution had subsided and the temperature had decreased, THF (30 mL) was added to the mixture. The reaction mixture was cooled to 15 °C, and freshly distilled tri-n-butyltin chloride (4.8 g, 14 mmol) was added to the activated magnesium over 10 min. Subsequently, geranyl bromide (14, 2.55 g, 11.7 mmol) in THF (10 mL) was added over 20 min at 0 °C. The reaction mixture was stirred for 45 min, during which it became dark grey and salts formed. The reaction was quenched by pouring the mixture into ice/water. It was extracted with ether (3 × 25 mL). The combined organic phases were washed with brine and dried with MgSO₄, and the solvents were evaporated in vacuo. Vacuum distillation gave the product 13 in 65% yield (3.2 g, 7.6 mmol); b.p. 50−155 °C at 0.8 Torr; n_D^{20} = 1.4953. ¹H NMR (CDCl₃, 300 MHz): δ = 5.32 (t, $^3J_{H-H}$ = 8.6 Hz,

1 H, CH), 5.10 (br. t, $^3J_{H-H}$ = 5.1 Hz, 1 H, CH), 2.00 (m, 6 H, 3 CH$_2$), 1.68 (s, 3 H, CH$_3$), 1.60 (s, 3 H, CH$_3$), 1.57 (s, 3 H, CH$_3$), 1.48 (m, 6 H, 3 CH$_2$), 1.32 (m, 12 H, 6 CH$_2$), 0.89 (m, 9 H, 3 CH$_3$) ppm. ^{13}C NMR (CDCl$_3$, 75 MHz): δ = 134.2 (C), 131.4 (C), 124.7 (CH), 122.9 (CH), 39.9 (CH$_2$), 29.2 (CH$_2$), 27.4 (CH$_2$), 27.1 (3 CH$_2$), 25.7 (3 CH$_3$), 13.7 (3 CH$_3$), 10.6 (3 CH$_2$), 9.4 (3 CH$_2$) ppm. MS (ESI): m/z = 428 [M + H].

Synthesis of 3-Demethoxy-3-hydroxyubiquinone-0 (20)

2-Acetyl-3-hydroxy-4,5-dimethoxytoluene: AlCl$_3$ (80 g, 0.60 mol) was added, as quickly as possible, to a vigorously stirred solution of 2,3,4-trimethoxytoluene (**7**, 50 g, 0.27 mol) in ether (800 mL). The temperature of the reaction mixture rose slowly to the reflux temperature of ether, despite cooling in an ice bath. After the exothermic effect had subsided, acetyl chloride (43 g, 0.55 mol) was added dropwise over about 20 min, whereupon the temperature of the reaction mixture again slowly increased to the reflux temperature of ether (no cooling was applied). When this exothermic effect had decreased, heating under reflux (50 °C in a warm water bath) was continued for another 3 h, after which conversion was complete (no starting compound was found by NMR analysis). After cooling towards room temp., the two-layer system was very cautiously poured onto ice (about 1 kg) with continuous swirling by hand. After separation of the ether layer, the water layer was saturated with NaCl and extracted with ether (4 × 100 mL). The combined ether fractions were subsequently extracted with a 1 M NaOH solution (4 × 100 mL). The combined water layers were cooled in ice and slowly acidified to pH = 2 by addition of concentrated HCl and placed on ice. After standing for 30 min, the solid was filtered off and dried between filtration paper overnight. A pale yellow solid was obtained in 64% yield (37 g, 0.18 mol); m.p. 87−89 °C. ^{1}H NMR (CDCl$_3$, 300 MHz): δ = 7.26 (s, 1 H, OH), 6.31 (s, 1 H, CH), 3.91 (s, 3 H, OCH$_3$), 3.86 (s, 3 H, OCH$_3$), 2.62 (s, 3 H, CH$_3$), 2.54 (s, 3 H, CH$_3$) ppm. ^{13}C NMR (CDCl$_3$, 75 MHz): δ = 204.3 (C=O), 156.6 (CO), 155.8 (CO), 135.9 (C), 135.5 (C), 118.2 (C), 106.8 (CH), 60.5 (OCH$_3$), 55.7 (OCH$_3$), 32.9 (CH$_3$), 24.4 (CH$_3$) ppm. MS (ESI): m/z = 210 [M], 195 [M − CH$_3$].

2,3-Dihydroxy-4,5-dimethoxytoluene (6): Sodium hydroxide (4.8 g, 0.12 mol) in water (50 mL) was added to a solution of 2-acetyl-3-hydroxy-4,5-dimethoxytoluene (21 g, 0.10 mol) in methanol (150 mL), whereupon a slight rise from room temp. to 30 °C was observed. Aqueous H$_2$O$_2$ (35%, 13 mL, 0.13 mol), further diluted with 30 mL of water, was then added dropwise at 25 °C over about 45 min. The temperature rose to about 50 °C. During the reaction the colour turned from light purple to dark purple. After the addition, the reaction temperature started to decrease. For a short period (5 min) the reaction mixture was heated under reflux (free flame), after which it was cooled to room temp. After acidification of the reaction mixture to pH = 6−7 by addition of a few drops of concentrated HCl, it was saturated with NaCl and subsequently extracted with dichloromethane (8 × 50 mL), the combined organic layers were dried with MgSO$_4$, and the solvents were evaporated in vacuo. An orange-brown solid **6** was obtained in 95% yield (17.5 g, 0.095 mol); m.p. 97−99 °C. ^{1}H NMR (CDCl$_3$, 300 MHz): δ = 6.27 (s, 1 H, OH), 6.25 (s, 1 H, CH), 5.00 (s, 1 H, OH), 3.85 (s, 3 H, OCH$_3$), 3.78 (s, 3 H, OCH$_3$), 2.20 (s, 3 H, CH$_3$) ppm. ^{13}C NMR (CDCl$_3$, 75 MHz): δ = 145.4 (COH), 145.3 (COH), 136.5 (CO), 136.4 (CO), 118.8 (C), 105.4 (CH), 61.0 (OCH$_3$), 55.7 (OCH$_3$), 15.4 (CH$_3$) ppm. MS (ESI): m/z = 184 [M], 169 [M − CH$_3$], 155 [M − CHO].

3-Demethoxy-3-hydroxyubiquinone-0 (20): V$_2$O$_5$ (50 mg, 0.3 mmol) was added at room temp to a vigorously stirred suspension of **6**

(17.5 g, 95 mmol) in 2% sulfuric acid (200 mL). A solution of Na-ClO$_3$ (6.0 g, 57 mmol) in water (50 mL) was added dropwise to this red-brown reaction mixture over about 10 min. No heat was evolved, but the colour of the reaction mixture turned dark red. Stirring was continued for about 6 h, after which the conversion was complete (according to NMR analysis). The non-homogenous mixture was poured into a saturated NaCl solution, and the aqueous phase was extracted with dichloromethane (5 × 50 mL). The combined organic fractions were dried with MgSO$_4$ and filtered, and the solvents were evaporated in vacuo. A dark red solid **20** was obtained, in a yield of 88% (14 g, 83 mmol). The very pure compound was obtained by crystallization from tetrachloromethane; m.p. 100−102 °C. ^{1}H NMR (CDCl$_3$, 300 MHz): δ = 6.55 (s, 1 H, OH), 6.41 (q, $^4J_{H-H}$ = 1.5 Hz, 1 H, CH), 4.09 (s, 3 H, OCH$_3$), 2.07 (d, $^4J_{H-H}$ = 1.5 Hz, 3 H, CH$_3$) ppm. ^{13}C NMR (CDCl$_3$, 75 MHz): δ = 191.5 (C=O), 191.4 (C=O), 162.8 (CO), 160.0 (CC), 151.2 (CO), 132.5 (CH), 60.3 (OCH$_3$), 14.9 (CH$_3$). HRMS (DIP): calcd. for C$_8$H$_8$O$_4$ 168.0423; found 168.044±0.003.

Synthesis of 3-Demethoxyubiquinone-0 (30)

3-Methoxy-2-cyclohexenone: Trimethyl orthoformate (10.6 g, 100 mmol) and a catalytic quantity of 37% HCl (0.2 mL) were added successively to a well-stirred solution of 1,3-cyclohexanedione (**12**, 7.5 g, 69 mmol) in methanol (100 mL), in which the air had been completely replaced by nitrogen. This yellow-orange homogenous reaction mixture was either heated under reflux for a period of about 45 min, or was stirred at room temp. for about 12 h. An orange-red clear solution resulted in either case, and this was now completely concentrated in vacuo. The remaining residue was distilled and gave the pure, colourless 3-methoxy-2-cyclohexenone (b.p. 68 °C at 0.6 mbar) in 95% yield (8.1 g, 64 mmol). n_D^{20} = 1.5072. ^{1}H NMR (CDCl$_3$, 300 MHz): δ = 5.38 (s, 1 H, CH), 3.71 (s, 3 H, CH$_3$), 2.44 (t, $^3J_{H-H}$ = 6.3 Hz, 2 H, CH$_2$), 2.36 (dd, $^3J_{H-H}$ = 6.3 Hz and 6.9 Hz, 2 H, CH$_2$), 2.01 (m, 2 H, CH$_2$) ppm. ^{13}C NMR (CDCl$_3$, 75 MHz): δ = 199.7 (C=O), 178.9 (CO), 101.9 (CH), 55.4 (OCH$_3$), 36.3 (CH$_2$), 28.5 (CH$_2$), 20.6 (CH$_2$) ppm. MS (ESI): m/z = 126 [M].

3-Methoxy-6-methyl-2-cyclohexenone (11): Under nitrogen, a BuLi solution in hexanes (1.6 M, 40 mL, 64 mmol) was added by syringe over 5 min, starting at −20 °C, to a stirred solution of diisopropylamine (7.0 g, 70 mmol) in THF (50 mL). After the exothermic effect had subsided (a few minutes), the light yellowish reaction mixture was cooled to −65 °C. A solution of 3-methoxy-2-cyclohexenone (8.1 g, 64 mmol) in THF (30 mL) was added dropwise over 10 min. Despite cooling, the temperature rose to −50 °C, and a yellow suspension was formed. After stirring for 20 min at −30 °C, the reaction mixture was again cooled to −65 °C, and a solution of iodomethane (10 g, 70 mmol) in THF (15 mL) was added over 5 min. After the exothermic reaction (a temperature rise to −50 °C occurred, despite cooling), the cooling bath was removed and stirring was continued for an additional 30 min at −30 °C (cooling now and then). The yellow/white suspension was quenched with a saturated aqueous ammonium chloride solution (100 mL). After separation of the organic layer, the water layer was extracted with ether (5 × 50 mL), the combined organic layers were washed with brine (1 × 40 mL) and dried with MgSO$_4$, and the solvents were evaporated in vacuo. A liquid was obtained, with a crude yield of 8.2 g. Distillation of this yielded the pure 3-methoxy-6-methyl-2-cyclohexenone (**11**, b.p. 66−68 °C at 0.4 Torr) as a colourless liquid in 88% yield (7.9 g, 56 mmol); m.p. 16 °C; n_D^{20} = 1.5002. ^{1}H NMR (CDCl$_3$, 300 MHz): δ = 5.35 (s, 1 H, CH), 3.69 (s, 3 H, OCH$_3$), 2.46 (m, 1 H, CH), 2.31 (m, 1 H, CH$_2$), 2.06 (ddd, $^3J_{H-H}$ = 4.8 Hz and 9.6 Hz, 2 H, CH$_2$), 1.70 (m, 1 H, CH$_2$), 1.15 (d, $^3J_{H-H}$ =

6.9 Hz, 3 H, CH$_3$) ppm. ^{13}C NMR (CDCl$_3$, 75 MHz): δ = 201.8 (C=O), 177.6 (CO), 101.4 (CH), 55.5 (OCH$_3$), 39.9 (CH), 29.0 (CH$_2$), 28.0 (CH$_2$), 15.1 (CH$_3$) ppm. MS (ESI): m/z = 140 [M], 112 [M − CO].

3-Methoxy-6-methyl-1-(trimethylsiloxy)-2,6-cyclohexadiene: A solution of lithium diisopropylamide was prepared first at −10 °C from diisopropylamine (6.6 g, 65 mmol) in THF (50 mL) and a BuLi solution in hexanes (1.6 M, 37.5 mL, 60 mmol) under nitrogen and with continuous stirring. At an internal temperature of −10 °C, a solution of 3-methoxy-6-methyl-2-cyclohexenone (**11**, 7.9 g, 56 mmol) in THF (30 mL) was added dropwise over 30 min. During this addition the temperature of the reaction mixture rose to about −5 °C despite continuous cooling (in an ice/salt bath). After the addition was complete, the clear yellow solution was stirred for an additional 15 min at 0 °C and cooled again to −10 °C, whereupon freshly distilled chlorotrimethylsilane (7.6 g, 70 mmol) was added in one portion. The cooling bath was removed and the reaction mixture was allowed to warm up to 10 °C over about 20 min. The reaction mixture was quenched with a saturated aqueous sodium carbonate solution (200 mL), and the organic layer was immediately separated and dried with Na$_2$SO$_4$. The water layer was extracted with ether (3 × 50 mL) and these ether fractions were added to the dried organic layer. After filtration and evaporation of the solvents in vacuo, 12.3 g of a light orange liquid resulted. Distillation gave the colourless silyl enol ether (b.p. 62 °C at 0.3 mbar) in 97% yield (11.5 g, 54.1 mmol). ^{1}H NMR (CDCl$_3$, 300 MHz): δ = 5.26 (s, 1 H, CH), 3.69 (s, 3 H, OCH$_3$), 1.85 (m, 4 H, 2CH$_2$), 1.43 (s, 3 H, CH$_3$), 0.85 [s, 9 H, Si(CH$_3$)$_3$] ppm. MS (ESI): m/z = 213 [M + H].

3-Methoxy-6-methylphenol (9): Activated MnO$_2$ (10.0 g, 115 mmol) was added in one portion to a solution of the silyl enol ether (11.5 g, 54.1 mmol) in ether (150 mL), whereupon a small rise in temperature from 18 to 25 °C was observed. This mixture was then stirred overnight (18 h), after which it was filtered through Hyflo Super Cell. The Hyflo layer was thoroughly washed with ether, and the combined ether fractions were dried with MgSO$_4$. After removal of the solvent in vacuo, the product was dissolved in THF (100 mL), and a 10% HCl solution (50 mL) was added to the stirred solution at room temp. The temperature rose from 20 to 30 °C, and the solution changed from a yellow clear solution to a yellow/white suspension. After the mixture had been stirred for 30 min (the temperature decreasing to 20 °C), ether (100 mL) was added, followed by a saturated aqueous NaCl solution (100 mL). The organic layer was separated from the water layer and the water layer was extracted with ether (5 × 50 mL). The combined organic phases were thoroughly dried with MgSO$_4$. Evaporation of the solvents in vacuo gave 6.9 g of an orange-red residue. Distillation gave the pure phenol **9** (b.p. 87 °C at 0.5 Torr) as a light yellow, viscous liquid in 90% yield (6.2 g, 45 mmol). n_D^{22} = 1.5428. ^{1}H NMR (CDCl$_3$, 300 MHz): δ = 6.98 (d, $^3J_{H-H}$ = 7.8 Hz, 1 H, CH), 6.39 (d, $^3J_{H-H}$ = 7.8 Hz, 1 H, CH), 6.38 (s, 1 H, CH), 5.62 (s, 1 H, OH), 3.71 (s, 3 H, OCH$_3$), 2.16 (s, 3 H, CH$_3$) ppm. ^{13}C NMR (CDCl$_3$, 75 MHz): δ = 158.6 (CO), 154.6 (COH), 131.1 (CH), 116.2 (CC), 105.6 (CH), 101.4 (CH), 55.2 (OCH$_3$), 14.9 (CH$_3$) ppm. MS (ESI): m/z = 138 [M], 107 [M − OCH$_3$].

3-Demethoxyubiquinone-0 (30): K$_2$HPO$_4$ (40 g, 0.22 mol) was dissolved in water (1 L) and the pH of this buffer was set at 6.5 with concentrated HCl. Potassium nitrosodisulfonate (Fremy's salt, 45 g) was dissolved in this buffer, and a solution of 3-methoxy-6-methylphenol (**9**, 6.2 g, 45 mmol) in ether (50 mL) was added. After stirring overnight (about 18 h) the layers were separated and the

water layer was extracted with dichloromethane (5 × 100 mL). The combined organic phases were washed with brine and dried with MgSO$_4$, and the solvents were evaporated in vacuo. The product was purified by silica gel chromatography (80% ether in PE). A yellow solid (**30**) was obtained, with a yield of 68% (4.7 g, 31 mmol). ^{1}H NMR (CDCl$_3$, 300 MHz): δ = 6.56 (q, $^4J_{H-H}$ = 1.5 Hz, 1 H, CH), 5.93 (s, 1 H, CH), 3.82 (s, 3 H, OCH$_3$), 2.07 (d, $^4J_{H-H}$ = 1.5 Hz, 3 H, CH$_3$) ppm. ^{13}C NMR (CDCl$_3$, 75 MHz): δ = 187.6 (C=O), 182.1 (C=O), 158.6 (CO), 146.8 (CC), 131.2 (CH), 107.5 (CH), 56.2 (OCH$_3$), 15.7 (CH$_3$). HRMS (DIP): calcd. for C$_8$H$_8$O$_3$ 152.0473; found 152.0461.

Synthesis of Ubiquinone-2 (1)

[1′-^{14}C]Ubiquinone-2 (1a) and Ubiquinone-2 (1): Freshly distilled BF$_3$−OEt$_2$ (1.4 g, 10 mmol) was added at −78 °C to a solution of ubiquinone-0 (**10**, 0.60 g, 3.3 mmol) in dichloromethane (50 mL) and stirring was continued for 5 min. Tri-n-butyl(geranyl)tin (**13**, 1.6 g, 3.7 mmol) in dichloromethane (20 mL) was added at −78 °C over 5 min. When the addition was complete, the cooling bath was removed and the reaction mixture was allowed to warm to room temp. After 2 h, a 10% HCl solution (35 mL) was added at 15 °C and the mixture was stirred for 5 min. The aqueous phase that separated was extracted with dichloromethane (3 × 15 mL), and the combined organic layers were washed with brine (2 × 20 mL) and concentrated in vacuo. The residual oil was dissolved in ether (50 mL) and in FeCl$_3$ (3.0 g, 18 mmol) in water (50 mL) and heated under reflux for 30 min. The organic layer was washed with brine (3 × 15 mL) and the combined organic layers were stirred with aqueous potassium fluoride (10%, 50 mL) for 30 min. The insoluble tin salts were removed by filtration through Hyflo, and the organic phase in the filtrate was washed with brine (3 × 10 mL), dried with MgSO$_4$ and concentrated in vacuo. The product **1** was purified by silica gel chromatography (80% ether in petroleum ether), which gave a yellow oil with a yield of 1.0 g (95%). ^{1}H NMR (CDCl$_3$, 600 MHz): δ = 5.03 (t, $^3J_{H-H}$ = 7.0 Hz, 1 H, 2′-CH), 4.93 (t, $^3J_{H-H}$ = 7.0 Hz, 1 H, 6′-CH), 3.99 and 3.98 (both s, 3 H, 5 and 6-OCH$_3$), 3.18 (d, $^3J_{H-H}$ = 7.0 Hz, 1 H, 1′-CH$_2$), 2.05 (m, 2 H, 5′-CH$_2$), 2.01 (s, 3 H, 3-CH$_3$), 1.96 (m, 2 H, 4′-CH$_2$), 1.73 (s, 3 H, 9′-CH$_3$), 1.65 (s, 3 H, 8′-CH$_3$), 1.57 (s, 3 H, 10′-CH$_3$). ^{13}C NMR (CDCl$_3$, 150 MHz): δ = 184.7 (4-CO), 183.9 (1-CO), 144.4 and 144.2 (both 5- and 6-CO), 141.7 (2-C), 138.8 (3-C), 137.5 (3′-C), 131.5 (7′-C), 124.0 (6′-CH), 118.9 (2′-CH), 60.8 (5- and 6-OCH$_3$), 39.7 (4′-CH$_2$), 26.6 (5′-CH$_2$), 25.6 (8′-CH$_3$), 25.3 (1′-CH$_2$), 17.6 (10′-CH$_3$), 16.2 (9′-CH$_3$), 11.9 (3-CH$_3$). HRMS (DIP): calcd. for C$_{19}$H$_{26}$O$_4$ 318.1831; found 318.1796.

Synthesis of 5-Demethoxy-5-hydroxyubiquinone-2 (2)

3-Acetoxy-2-methoxy-5-methyl-1,4-benzoquinone (5): A solution of 3-demethoxy-3-hydroxyubiquinone-0 (**20**, 14 g, 83 mmol) in acetic anhydride (100 mL) was heated under reflux for 15 min, whereupon the reddish solution turned yellow. According to TLC, the conversion was complete. After distillation of most of the (excess of) acetic anhydride at atmospheric pressure, the last traces were removed by evaporation with toluene under vacuum pressure. The product was dissolved in ether (100 mL) and pre-purified by a flash column on silica gel. This yielded an orange solid in about 95% yield (16.7 g, 79.5 mmol). The pure component **5** was obtained by crystallization from ether/hexane as orange-yellow crystals; m.p. 92−94 °C. ^{1}H NMR (CDCl$_3$, 300 MHz): δ = 6.43 (s, 1 H, CH), 4.01 (s, 3 H, OCH$_3$), 2.26 (s, 3 H, CH$_3$), 1.98 (s, 3 H, CH$_3$) ppm. ^{13}C NMR (CDCl$_3$, 75 MHz): δ = 184.2 (C=O), 182.7 (C=O), 181.1 (C=O), 167.9 (CO), 147.5 (CO), 144.5 (CC), 131.1 (CH), 60.2 (OCH$_3$), 20.2 (CH$_3$), 15.3 (CH$_3$) ppm. MS (ESI): m/z = 210 [M], 195 [M − CH$_3$].

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[1′-¹⁴C]-3-Acetoxy-6-geranyl-2-methoxy-5-methyl-1,4-benzoquinone and 3-Acetoxy-6-geranyl-2-methoxy-5-methyl-1,4-benzoquinone: Freshly distilled BF₃·OEt₂ (2.8 g, 20 mmol) in dichloromethane (10 mL) was added over 3 min under nitrogen at −70 °C to a solution of 3-acetoxy-2-methoxy-5-methyl-1,4-benzoquinone (**5**, 1.1 g, 5.2 mmol) in dichloromethane (30 mL). This was followed by the addition over 20 min and at −70 °C of distilled tri-*n*-butyl(geranyl)-tin (**12**, 2.6 g, 6.0 mmol) in dichloromethane (15 mL). After stirring for 3 h at −70 °C, the reaction mixture was poured into an ice-cold HCl solution (10%, 60 mL), directly followed by addition of brine (30 mL). After stirring for 10 min, this mixture was extracted with dichloromethane (2 × 25 mL). The organic phase was concentrated in vacuo and dissolved in ether (40 mL), and FeCl₃ (5 g) in water (25 mL) was added. After the mixture had been stirred overnight, the layers were separated, the water phase was extracted with ether (1 × 30 mL), and the combined organic layers were concentrated in vacuo. The residue was dissolved in ether (25 mL), and KF (5 g) in water (25 mL) was added. After stirring for 90 min, the mixture was filtered through Hyflo, the water phase was extracted with ether (4 × 15 mL), the combined organic phases were dried with MgSO₄ and filtered, and the solvents were evaporated in vacuo. The product was purified by silica gel chromatography (100% petroleum ether → 40% ether in petroleum ether), which gave 1.05 g of a yellow oil (75%, 3.8 mmol). ¹H NMR (CDCl₃, 300 MHz): δ = 5.03 (t, $^3J_{H-H}$ = 6.6 Hz, 1 H, CH), 4.93 (t, $^3J_{H-H}$ = 6.2 Hz, 1 H, CH), 4.06 (s, 3 H, OCH₃), 3.20 (d, $^3J_{H-H}$ = 6.6 Hz, 2 H, CH₂), 2.32 (s, 3 H, CH₃), 2.03 (m, 2 H, CH₂), 2.00 (s, 3 H, CH₃), 1.99 (m, 2 H, CH₂), 1.73 (s, 3 H, CH₃), 1.65 (s, 3 H, CH₃), 1.58 (s, 3 H, CH₃) ppm. ¹³C NMR (CDCl₃, 75 MHz): δ = 182.5 (C=O), 181.3 (C=O), 178.1 (C=O), 168.0 (CO), 147.1 (CO), 141.7 (C), 139.2 (C), 137.5 (C), 131.3 (C), 123.8 (CH), 118.4 (CH), 60.1 (OCH₃), 39.4 (CH₂), 26.3 (CH₂), 25.4 (CH₂), 25.2 (CH₂), 20.1 (CH₃), 17.4 (CH₃), 16.1 (CH₃), 11.7 (CH₃) ppm. MS (ESI): *m/z* = 346 [M].

[1′-¹⁴C]-5-Demethoxy-5-hydroxyubiquinone-2 (2a) and 5-Demethoxy-5-hydroxyubiquinone-2 (2): Nitrogen gas was passed through a solution of 3-acetoxy-6-geranyl-2-methoxy-5-methyl-1,4-benzoquinone (1.05 g, 3.8 mmol) in THF (40 mL). A solution of K₂CO₃ (1.7 g, 15 mmol) in water (15 mL) was added dropwise at 0 °C over 10 min, during which the solution turned dark purple. According to TLC analysis, deacetylation was complete after stirring for 1 h at 5−10 °C. The mixture was acidified with concentrated HCl until the mixture turned yellow. After separation, the water layer was extracted with ether (4 × 10 mL), the combined ether fractions were dried with MgSO₄ and filtered, and the solvents were evaporated in vacuo. According to NMR spectroscopic data, this compound had a purity of about 97%, but it was further purified by the following method, continuously under the exclusion of air. The product was dissolved in ether (50 mL) and this was extracted with a nitrogen-flushed NaOH solution (0.02 M, 4 × 15 mL). The purple water layer was washed with ether (3 × 10 mL), and the water layer was acidified with concentrated HCl until the colour changed from orange to red. This was extracted with ether (5 × 10 mL), dried with MgSO₄, filtered, and concentrated in vacuo. This resulted in an 84% yield of an orange oil of **2**, with a purity over 99% (0.75 g, 2.46 mmol). ¹H NMR (CDCl₃, 600 MHz): δ = 6.50 (br. s, 1 H, 5-OH), 5.03 (t, $^3J_{H-H}$ = 7.0 Hz, 1 H, 2′-CH), 4.92 (t, $^3J_{H-H}$ = 6.6 Hz, 1 H, 6′-CH), 4.07 (s, 3 H, 6-OCH₃), 3.20 (d, $^3J_{H-H}$ = 7.0 Hz, 2 H, 1′-CH₂), 2.06 (m, 2 H, 5′-CH₂), 2.04 (s, 3 H, 3-CH₃), 1.97 (m, 2 H, 4′-CH₂), 1.74 (s, 3 H, 9′-CH₃), 1.65 (s, 3 H, 8′-CH₃), 1.58 (s, 3 H, 10′-CH₃) ppm. ¹³C NMR (CDCl₃, 150 MHz): δ = 185.2 (4-CO), 183.2 (1-CO), 143.4 (6-CO), 139.3 (5-COH), 137.8 (3′-C), 137.1 (2-C), 136.2 (3-C), 131.6 (7′-C), 124.0 (6′-CH), 118.7 (2′-CH), 60.3 (6-OCH₃), 39.7 (4′-CH₂), 26.5 (5′-CH₂), 25.7 (8′-

CH₃), 25.4 (1′-CH₂), 17.7 (10′-CH₃), 16.3 (9′-CH₃), 11.6 (3-CH₃). HRMS (DIP): calcd. for C₁₆H₂₄O₄ 304.1675; found 304.166±0.003. IR: ṽ = 3366 (OH), 1639 (C=O), 1617 (C=O) cm⁻¹.

Synthesis of 5-Demethoxyubiquinone-2 (3)

***rac*-1,4,4a,8aα-Tetrahydro-7-methoxy-4aα-methyl-1aα,4α-methano-naphthaline-5,8-dione (8):** Freshly distilled cyclopentadiene (3.4 mL, 51 mmol) was added under nitrogen to a solution of **30** (1.3 g, 8.6 mmol) in THF (30 mL). The nitrogen flow was stopped and the reaction mixture was stirred for 20 h, after which it was concentrated in vacuo. The Diels−Alder product was purified by silica gel chromatography (40% ether in petroleum ether). A cream-coloured solid **8** was obtained, with a yield of 1.6 g (87%, 7.5 mmol); m.p. 102 °C. ¹H NMR (CDCl₃, 300 MHz): δ = 6.16 (dd, $^3J_{H-H}$ = 2.9 Hz, 5.6, 1 H, CH), 6.00 (dd, $^3J_{H-H}$ = 2.9 Hz, 5.6, 1 H, CH), 5.85 (s, 1 H, CH), 3.74 (s, 3 H, OCH₃), 3.45 (m, 1 H, CH), 3.08 (m, 1 H, CH), 2.89 (d, $^3J_{H-H}$ = 3.9 Hz, 1 H, CH), 1.68 + 1.53 (br. dd, $^2J_{H-H}$ = 45, $^3J_{H-H}$ = 1.3 Hz, 2 H, CH₂), 1.49 (s, 3 H, CH₃) ppm. ¹³C NMR (CDCl₃, 75 MHz): δ = 202.8 (C=O), 194.9 C=O), 163.2 (CO), 139.3 (CC), 134.7 (CH), 114.7 (2XCH), 57.8 (OCH₃), 57.0 (CH), 54.1 (CH), 50.2 (CH), 47.1 (CH₂), 27.2 (CH₃) ppm. MS (ESI): *m/z* = 218.

[1′-¹⁴C]5-Demethoxyubiquinone-2 (3a) and 5-Demethoxyubiquinone-2 (3): Commercially available *t*BuOK (0.67 g, 6.0 mmol) was dissolved in a 4:1 mixture of *tert*-butyl alcohol and toluene (10 mL) and cooled to 0 °C. A mixture of the Diels−Alder adduct **8** (0.66 g, 3.0 mmol) and geranyl bromide (**14**), dissolved in *tert*-butyl alcohol/toluene (4:1 mixture, 5 mL), was added dropwise. The reaction mixture was stirred for 80 min with ice cooling (0−5 °C), after which it was poured into an aqueous saturated ammonium chloride solution. The water layer was extracted with ether (3 × 10 mL), the combined organic fractions were washed with brine and dried with MgSO₄, and the solvents were evaporated in vacuo. The product was purified by silica gel chromatography (50% ether in petroleum ether) and yielded 56% of the product (0.40 g, 1.13 mmol). The cyclopentadiene group was removed by heating under reflux in toluene (30 mL). According to TLC, cleavage was complete after 60 min. After evaporation of toluene, the pure product was obtained as a yellow oil in quantitative yield (0.34 g, 1.1 mmol). ¹H NMR (CDCl₃, 600 MHz): δ = 5.88 (s, 1 H, 5-CH), 5.03 (t, $^3J_{H-H}$ = 7.0 Hz, 1 H, 2′-CH), 4.94 (t, $^3J_{H-H}$ = 7.0 Hz, 1 H, 6′-CH), 3.80 (s, 3 H, 6-OCH₃), 3.22 (d, $^3J_{H-H}$ = 7.0 Hz, 2 H, 1′-CH₂), 2.04 (m, 2 H, 5′-CH₂), 2.04 (s, 3 H, 3-CH₃), 1.95 (m, 2 H, 4′-CH₂), 1.74 (s, 3 H, 9′-CH₃), 1.65 (s, 3 H, 8′-CH₃), 1.57 (s, 3 H, 10′-CH₃) ppm. ¹³C NMR (CDCl₃, 150 MHz): δ = 187.7 (4-CO), 181.8 (1-CO), 158.3 (6-CO), 141.8 (3-C), 141.3 (2-C), 137.6 (3′-C), 131.5 (7′-C), 124.0 (6′-CH), 118.8 (2′-CH), 107.0 (5-CH), 56.1 (6-OCH₃), 39.6 (4′-CH₂), 26.5 (5′-CH₂), 25.7 (8′-CH₃), 25.2 (1′-CH₂), 17.6 (10′-CH₃), 16.3 (9′-CH₃), 12.1 (3-CH₃). HRMS (DIP): calcd. for C₁₈H₂₄O₃ 288.1725; found 288.1707. IR: ṽ = 1648 (C=O), 1608 (C=O) cm⁻¹.

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