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Synthesis of Ubiquinones-3 Specifically Labelled with ^{13}C at C(5)- or C(6)- Positions

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Abstract: (5-^{13}C) and (6-^{13}C) ubiquinones-3 (**20a** and **20b**) were synthesised from (1-^{13}C) trichloroacetic acid (**8a**) in 12 steps. The key step was a *Diels Alder* reaction between 2,5-bis (trimethylsilyloxy)-3-methylfuran (**2**) and (2-^{13}C) 2-bromo-1,1-dichloroethylene (**4a**) to afford an equimolecular mixture of (3-^{13}C) 3-bromo-2-chloro-5-methylbenzoquinone (**6a**) and (2-^{13}C) 2-bromo-3-chloro-5-methylbenzoquinone (**7a**) which was easily separated by HPLC under the hydroquinone form. The labelled positions were assigned on the basis of ^1H-NMR and the two intermediates were separately elaborated to the corresponding (5-^{13}C) and (6-^{13}C) ubiquinones-3.

INTRODUCTION

Quinones labelled with stable isotopes are useful tools for various biophysical studies using FTIR[1], ENDOR[2], EPR[3], NMR[4] techniques as analytical methods. In our program directed towards the caracterization of photosynthetic reaction centers by FTIR difference spectroscopy, we developed the synthesis of ubiquinones-3 specifically labelled with ^{13}C at positions C(5) or C(6).

X-ray data of the photosynthetic reaction center of purple bacteria showed two quinones Q_A and Q_B which are known to exhibit different mid-point redox potentials and to play different roles[5]. Q_A is strongly bound to the protein and acts as a one-electron acceptor in contrast to Q_B which is weakly bound and operates as a two-electron gate. Recently FTIR differentiel spectroscopy studies have shown conclusive results concerning the strength of the hydrogen bonds[1]. By using ubiquinones labelled at the C(1) or C(4) positions strong interactions could be demonstrated only for the carbonyl at the C(4) position in the case of Q_A. Loose interactions for both carbonyls at C(1) and C(4) could be shown for the ubiquinone located in the Q_B site. In order to obtain additional structural informations concerning the positioning of the quinones and their interactions within the binding sites we considered to extend our studies with the same technique by using two ubiquinones specifically labelled at C(5)

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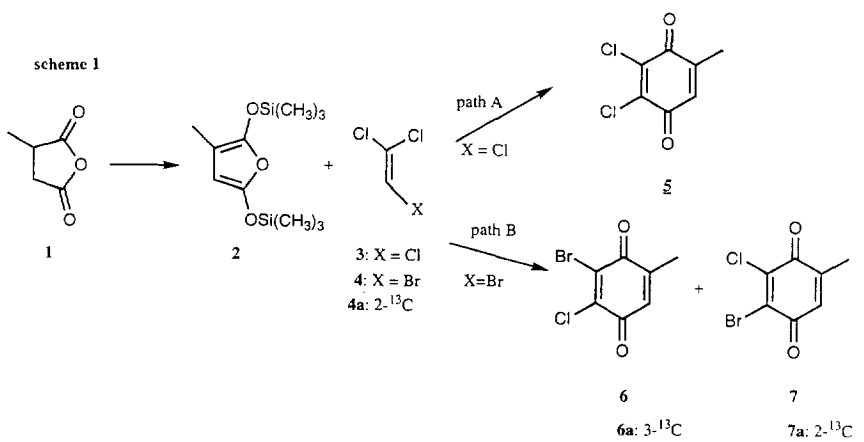
and C(6). These compounds could be useful probes to get informations about the conformation of the two methoxy groups at positions C(5) and C(6) when these ubiquinones are located in the protein niche. Indeed, the redox properties of the ubiquinones seem to be directly related to the orientation of the methoxy groups with respect to the quinone ring plane[6], rendering this study important for the comprehension of the photosynthetic pathway.

RESULTS AND DISCUSSION

The strategy for the synthesis of the ubiquinones **20a** and **20b** is based on a HPLC separation of an advanced intermediate which allows, after identification of the labelled positions by ^{1}H-NMR, the elaboration of the final ubiquinone with isotopic enrichments higher than 95% .

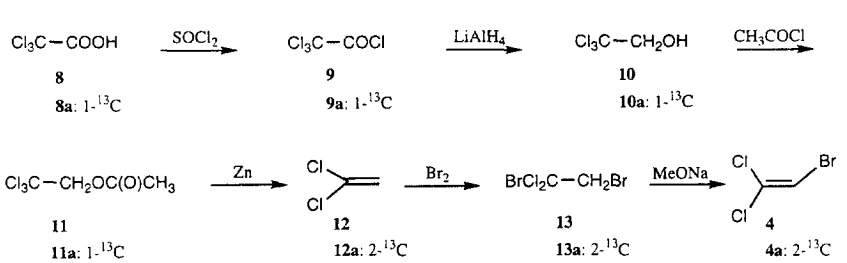
The synthesis of regiospecifically labelled quinones involves commercially available labelled starting compounds, which are converted into the final products in high yield and with total control of the labelled position. The synthesis is based on a procedure described previously by *Rüttiman* and *Lorenz*[7] using 2-bromo-1,1-dichloroethylene (**4**) (Scheme 1, path B), instead of trichloroethylene (**3**) (Scheme 1, path A), which led to a mixture of 2-bromo-3-chloro and 3-bromo-2-chloro regioisomers **6** and **7**. Using the labelled dienophile **4a**, the *Diels Alder* reaction afforded the regioisomers **6a** and **7a**, specifically labeled on C(3)- and C(2)- positions and their separation was performed by HPLC under the hydroquinonic form.

scheme 1



Synthesis of (2-^{13}C) 2-Bromo-1,1-dichloroethylene: 2-Bromo-1,1-dichloroethylene (**4a**) specifically labelled at the C(2)-position could be prepared from commercially available trichloroacetic acid (**8a**) labelled at C(1)-position in 6 steps (Scheme 2). However, the unlabelled dienophile **4** was more directly prepared from 1,1 dichloroethylene **12** in 2 steps.

scheme 2



We observed that reduction of trichloroacetic acid (**8**) to the alcohol **10** with $LiAlH_4$ gave a very low yield[8]. Also attempts to reduce the corresponding methyl ester led to unsatisfactory results. The transformation of **8** into acid chloride **9** could be achieved with thionyl chloride in 80% yield, after distillation. Reduction of **9** in Et_2O with $LiAlH_4$ led in 44% yield to a mixture of (1-^{13}C) trichloroethanol (**10a**) (83%) and (1-^{13}C) dichloroethanol (17%). At this stage the mixture could not be separated and was acetylated with acetyl chloride to give the two acetates in 90% yield[9]. Treatment with zinc afforded the pure (2-^{13}C) dichloroethylene (**12a**) in 50% yield, after distillation. Bromine addition at 0°C afforded **13a** in 66% yield, after distillation. 2-Bromo-1,1-dichloroethylene specifically labelled at the C(2)-position (**14a**) was obtained after treatment of (2-^{13}C) 1,2-dibromo-1,1-dichloroethane (**13a**) with sodium methoxide in 78% yield, after distillation[10].

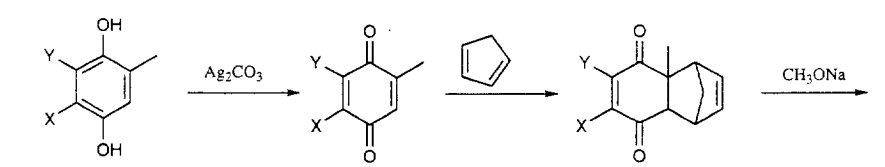
Synthesis of (5-^{13}C) and (6-^{13}C) ubiquinones-3: 2,5-Bis (trimethylsilyloxy)-3-methylfuran (**2**) was obtained from 2-methylsuccinic anhydride (**1**), in quantitative yield, by a known procedure[11] and was submitted to a *Diels Alder* reaction with the (2-^{13}C) 2-bromo-1,1-dichloroethylene (**4a**) in presence of pyridine at 110°C for 72 h in a sealed glass tube (Scheme 1).

After methanolysis, a mixture of two regioisomers (3-^{13}C) 3-bromo-2-chloro-5-methyl-1,4-benzoquinone (**6a**) and (2-^{13}C) 2-bromo-3-chloro-5-methyl-1,4-benzoquinone (**7a**) was obtained. Attempts to separate this mixture by HPLC on different supports failed. Therefore they were reduced in quantitative yield by sodium dithionite into the phenolic form **14a** and **15a** which could be easily separated by HPLC on a silicagel column, with pentane/ethyl acetate/acetic acid 97.5: 2.5: 0.5 as eluent. The two regioisomers **14a** and **15a** (order of elution) were obtained in equal amount (5 % overall yield). The low yield observed during the *Diels Alder* step was probably due to the low quantities engaged in this reaction, a control experiment carried out on a 5 g scale on unlabelled material gave a 53% yield[12].

Each regioisomer was analysed by ^1H-NMR, the relative position between H-C(6) and the carbone 13 was assigned on the basis of the signal corresponding to H-C(6) which was a doublet for **14a** due to a ^3J(H-C(6), $^{13}C(2)$) coupling and a singlet (no coupling) for **15a**.

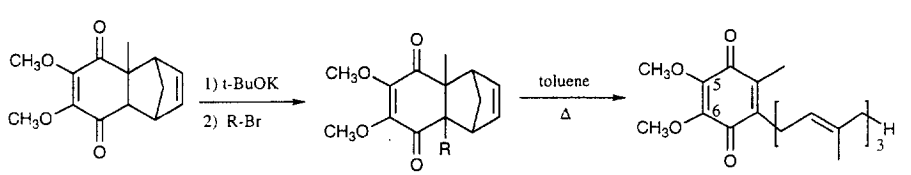
Subsequently, the two hydroquinones were treated separately, using the same reaction sequence, to afford the desired (5-^{13}C) and (6-^{13}C) ubiquinones-3 (**20a**) and (**20b**) respectively (Scheme 3).

scheme 3



14: X = Cl, Y = Br **14a:** 3-^{13}C **6:** X = Cl, Y = Br **6a:** 3-^{13}C **16:** X = Cl, Y = Br **16a:** 3-^{13}C

15: X = Br, Y = Cl **15a:** 2-^{13}C **7:** X = Br, Y = Cl **7a:** 2-^{13}C **17:** X = Br, Y = Cl **17a:** 7-^{13}C



18
18a: 6-^{13}C
18b: 7-^{13}C

R= farnesyl: **19**
19a: 6-^{13}C
19b: 7-^{13}C

20
20a: 5-^{13}C
20b: 6-^{13}C

Each hydroquinone **14a** and **15a** was oxidised by Ag_2CO_3 to regenerate the corresponding quinones **6a** and **7a** in quantitative yield[15]. According to the procedure of *Rüttiman* and *Lorenz*[7], quinones **6a** and **7a** were treated with cyclopentadiene to afford the two adducts **16a** and **17a** in quantitative yield. The halogen atoms were substituted by methoxy groups upon treatment with sodium methoxide to give, after HPLC purification **18a** and **18b** in 27% and 48% yields respectively. Alkylation of **18a** and **18b** with freshly distilled farnesyl bromide gave, after HPLC purification **19a** and **19b** in 29% and 23% yields. The *retro-Diels Alder* reaction finally afforded the title compounds **20a** (76%) and **20b** (85%).

These two labelled compounds **20a** and **20b** are currently studied by FTIR differential spectroscopy under light induced conditions in order to rely the structural informations to the redox properties of both Q_A and Q_B ubiquinones in the photosynthetic reaction center sites.

EXPERIMENTAL

General. All reactions were first optimised on unlabelled material and then carried out on the corresponding labelled compounds. The caracterisations of all new unlabelled compounds are described in references 14,17,19,20 and the labelled compounds in the experimental part. (1-^{13}C) trichloroacetic acid (isotopic enrichment: 99%) was obtained from *Isotec* France. HPLC analysis were performed on a *Merck* system, and the HPLC purifications on a *Dupont* system. ^{1}H-NMR spectrum were recorded in $CDCL_3$ at 300MHz and ^{13}C-NMR at 75 MHz on a *Bruker* AM 400.

(1-^{13}C) Trichloroacetyl chloride (9a), was prepared by adopting a known procedure[13]. Thionyl chloride (4.7 ml, 64.4 mmol) was added dropwise to a suspension of (1-^{13}C) trichloroacetic acid (**8a**) (10 g, 61 mmol) in DMF (0.49 ml, 6.3 mmol) and heated at 85-90°C for 2 h 50. The reaction mixture was distilled at atmospheric pressure to give 8.5 g of **9a** (yield: 75%) as a colorless oil which was directly used in the next step.

(1-^{13}C) 2,2,2-Trichloroethanol (10a), was prepared by adopting the procedure described in ref.8. To a suspension of $LiAlH_4$ (2.09 g, 55 mmol) in diethyl ether (40 ml), (1-^{13}C) 2,2,2-trichloroacetyl chloride (**9a**) (8.5 g, 46.4 mmol) was added dropwise such as the reflux of diethyl ether was maintained. At the end of the addition, the reaction mixture was stirred for 30 min at room temperature and cooled at -20°C in an alcohol-dry ice bath. Successively, 2 ml of cold water and 50 ml of a 10% solution of sulfuric acid were added. The crude mixture was extracted with diethyl ether (3x50 ml) and the combined extracts were dried over magnesium sulfate. Diethyl ether was removed and the crude mixture distilled under reduced pressure (20 Torr/66-68°C) to give 4.04 g (yield: 58%) of an inseparable mixture of (1-^{13}C) 2,2,2-trichloroethanol (**10a**) (83%) and (1-^{13}C) 2,2-dichloroethanol (17%). ^{1}H NMR of **10a**: 2.94 [*td*, *J*(H-C(1),OH) = 7.7, *J*(OH,^{13}C(1)) = 3.3, OH], 4.13 [*dd*, *J*(H-C(1),^{13}C(1) = 151, *J*(OH, H-C(1)) = 3.3, $^{13}CH_2$]; ^{13}C NMR: 76.0 ^{13}C(1).

(1-^{13}C) 2,2,2-Trichloroethyl acetate (11a), was prepared by adopting the procedure described in ref. 9. Acetyl chloride (6.4 ml, 90 mmol) was added dropwise to 3.6 g of the mixture of (1-^{13}C) 2,2,2-trichloroethanol (**10a**) (83%, 24.1 mmol) and of (1-^{13}C) 2,2-dichloroethanol (17%) obtained previously. The solution was stirred at 20°C overnight and refluxed for 4 h. The excess of acetyl chloride was removed and the crude mixture was distilled under reduced presssure (10 Torr/65°C) to give 4.61 g (yield: 87%) of an inseparable mixture of (1-^{13}C) 2,2,2-trichloroethyl acetate (**11a**) (83%) and (1-^{13}C) 2,2 dichloroethyl acetate (17%) as a colorless oil. ^{1}H NMR of **11a**: 2.16 (*s*, CH_3), 4.68 [*d*, *J*(H-C(1), ^{13}C(1) = 153, $^{13}CH_2$]; ^{13}C NMR: 73.7 ^{13}C(1).

(2-^{13}C) 1,1-Dichloroethylene (12a), was prepared by adopting the procedure described in ref. 9. A suspension of zinc powder (4.6 g,70 mmol) in ethanol (5 ml) was heated at 75-80°C and a solution of ethanol (5 ml) containing 4 g of the mixture of (1-^{13}C) 2,2,2-trichloroethyl acetate (**11a**) (83%, 20.8 mmol) and (1-^{13}C) 2,2 dichloroethyl acetate (17%) was added slowly. The reaction mixture was then heated at 95-100°C for 1 h. The crude mixture was distilled at atmospheric pressure and 0.99 g of pure

12a was collected at 36-44°C as a colorless oil (yield: 59%). ^1H NMR: 5.49 [*d*, *J*(H-C(2), $^{13}C(2)$ = 167, $^{13}CH_2$)]; ^{13}C NMR: 115.4 $^{13}C(2)$.

(2-^{13}C) 1,2-Dibromo-1,1-dichloroethane (13a),was prepared by adopting the procedure described in ref. 10. (2-^{13}C) 1,1-dichloroethylene (**12a**) (0.98 g, 10 mmol) was cooled at 0°C, anhydrous bromine (0.52 ml, 10 mmol) was added dropwise and the reaction mixture was stirred at 20°C for 30 min. The crude was distilled to give 1.7 g of **13a** (12 Torr/60-62°C) (yield: 66%). ^1H NMR: 4.40 [*d*, *J*(H-C(2), $^{13}C(2)$ = 158, $^{13}CH_2$]; ^{13}C NMR: 48.4 $^{13}C(2)$.

(2-^{13}C) 2-Bromo-1,1-dichloroethylene (4a), was prepared by adopting the procedure described in ref. 10. A solution of (2-^{13}C) 1,2-dibromo-1,1-dichloroethane (**13a**) (1.7 g, 6.6 mmol) in ethanol (4 ml) was cooled at 5°C and a solution of NaOMe (5.4 mmol of Na in 2.5 ml of methanol) was added dropwise. After 30 min at room temperature, water (10 ml) was added. The oily phase was separated. The aqueous phase was extracted with dichloromethane (2x5 ml). The oil and the organic extracts were combined, dried over Na_2SO_4 and distilled to give 0.92 g (20 Torr/26°C) of **14a** as a colorless oil (yield: 78%). ^1H -NMR: 6.61 [*d*, *J*(H-C(2), $^{13}C(2)$ = 201, $^{13}CH_2$]; ^{13}C NMR: 104.2 $^{13}C(2)$.

(3-^{13}C) 3-Bromo-2-chloro-5-methylbenzene-1,4-diol (14a) and (2-^{13}C) 2-bromo-3-chloro-5-methylbenzene-1,4-diol (15a). A sealed glass tube containing (2-^{13}C) 2-bromo-1,1-dichloroethylene (**4a**) (0.8 g, 4.5 mmol), 2,5-bis (trimethylsilyloxy)-3-methylfuran (**2**) (1.6 g, 5.8 mmol) and pyridine (0.33 ml, 4 mmol), was heated at 110°C for 72 h. The reaction mixture was treated with methanol (10 ml) at 60°C for 30 min and evaporated *in vacuo*. The residue was extracted with diethyl ether (3x10 ml) and to the combined extracts was added a 10% solution of sodium dithionite (20 ml). The mixture was vigourously stirred for 30 min at room temperature. The organic phase was evaporated *in vacuo* to give a crude mixture of **14a** and **15a** as a white solid. The hydroquinones were separated by HPLC on silicagel (pentane/ethyl acetate/acetic acid 97.5: 2.5: 0.5) which gave 32.8 mg of **14a** and 22 mg of **15a** (yield: 5 %).**14a**: ^1H NMR: 2.25 (*s*, CH_3), 5.8 (*br. s*, HO-C(1,4)), 6.82 (s, H-C(6)); ^{13}C NMR: 109.3 $^{13}C(3)$. **15a:** ^1H NMR: 2.22 (*s*, CH_3), 5.26, 5.14 (2*s*, HO-C(1), HO-C(4)), 6.79 [*d*, *J*(H-C(6),$^{13}C(2)$) = 9.1, H-C(6)]; ^{13}C NMR: 106.3 $^{13}C(2)$. (for **14** and **15** see ref.14).

(3-^{13}C) 3-Bromo-2-chloro-5-methyl-1,4-benzoquinone (6a),was prepared by adopting the procedure described in ref. 15. **14a** (22 mg, 0.091 mmol) was dissolved in benzene (5 ml) and Ag_2CO_3 (134 mg, 0.48 mmol) and $MgSO_4$ (134 mg, 1.11 mmol) were added. The mixture was stirred for 4 h at 20°C. The salt was removed by filtration on a pad of celite, washed with benzene (2x10 ml) and the combined organic extracts were evaporated *in vacuo* to give pure **6a** as a yellow solid in quantitative yield. ^1H NMR : 2.15 [*d*, *J*(CH_3, H-C(6) = 1.6, CH_3), 6.78 (*q*, H-C(6)); ^{13}C NMR : 135.5 $^{13}C(3)$.(for **6** see ref.16).

(2-^{13}C) 2-Bromo-3-chloro-5-methyl-1,4-benzoquinone (7a), was prepared by the same procedure as described for **6a**. Starting from 32 mg of **15a**, **7a** was obtained in quantitative yield. ^1H NMR : 2.15 [*d*, *J*(CH_3, H-C(6) = 1.6, CH_3], 6.78 [*qd*, *J*(H-C(6),$^{13}C(2)$) = 7.8, H-C(6)]; ^{13}C NMR : 136.3 $^{13}C(2)$. To a solution of **6a** (21 mg, 0.088 mmol) in methanol (2 ml), cooled at 0°C, was added freshly distilled cyclopentadiene (0.2 ml, 2.4 mmol). The mixture was stirred for 30 min at 0°C and at room temperature for 4 h. The solvent was evaporated *in vacuo* and the crude product was purified by HPLC on silicagel (hexane/ethyl acetate 9:1) to give 23.5 mg of **16a** (yield : 87%). ^1H NMR : 1.53 (*s*, CH_3), 1.58,1.72 (*br.s*, *J* = 9.4, CH_2), 3.03 [*d*, *J*(H-C(1), H-C(8a) = 3.8, H-C(8a)], 3.16, 3.49 (*br.s*, H-C(1), H-C(4)), 6.03, 6.15 [*dd*, *J*(H-C(2), H-C(3) = 6.3, H-C(2), H-C(3)]; ^{13}C NMR : 142.0 $^{13}C(6)$. (for **16** see ref.17).

(7-^{13}C) (1α,4α,4aβ,8aβ) 7-Bromo-6-chloro-1,4,4a,8a-tetrahydro-4a-methyl-1,4-methanonaphtalene-5,8-dione (17a),was prepared by the same procedure as described above. Starting from 32 mg of **7a**, 35 mg of **17a** was obtained (yield : 85%). ^1H NMR : 1.53 (*s*, CH_3), 1.57,1.72 (*br.s*, *J* = 9.4, CH_2), 3.07 [*dd*, *J*(H-C(8a),$^{13}C(7)$) = 2.7, *J*(H-C(8a),H-C(1) = 3.5, H-C(8a)]. 3.15, 3.47 (*br.s*, H-C(1), H-C(4)), 6.03, 6.15 [*dd*, *J*(H-C(2), H-C(3) = 5.6, H-C(2), H-C(3)]; ^{13}C NMR : 142.1 $^{13}C(7)$. (for **17** see ref.18).

(6-^{13}C) (1α,4α,4aβ,8aβ) Tetrahydro-6,7-dimethoxy-4a-methyl-1,4-methanonaphtalene-5,8-dione (18a), was prepared by the same procedure as described above. To a cold solution of **16a** (23 mg, 0.076 mmol) in toluene (3 ml) was added a solution of NaOMe (4 M, 0.2 mmol). The mixture was stirred for 1 h at 5°C and for 2 h at 20°C. The reaction mixture was neutralised by acetic acid. 10 ml of water was added and the mixture was extracted by diethyl ether (3x5 ml). The combined organic extracts were evaporated *in vacuo*. The crude product was purified by HPLC on silicagel (hexane/ethyl acetate 9:1) to give 5.2 mg of **18a** (yield : 27%). ^{1}H NMR : 1.47 (*s*, CH_3), 1.53, 1.65 (*br.d*, *J* = 9, CH_2), 2.82 [*d*, *J*(H-C(1), H-C(8a) = 3.8, H-C(8a)], 3.08, 3.42 (*s*, H-C(4), H-C(1)), 3.91 (*d*, *J*(CH_3O-C(6),^{13}C(6)) = 3.1, CH_3O-C(6)], 3.92 (*s*, CH_3O-C(7)), 6.02, 6.15 (*dd*, *J* = 5.6, H-C(2),H-C(3)]; ^{13}C NMR : 150.3 ^{13}C(6).

(7-^{13}C) (1α,4α,4aβ,8aβ) Tetrahydro-6,7-dimethoxy-4a-methyl-1,4-methanonaphtalene-5,8-dione (18b), was prepared by the same procedure as described above. Starting from 32 mg of **17a**, 12.8 mg of **18b** was obtained (yield : 48%). ^{1}H NMR : 1.47 (*s*, CH_3), 1.53, 1.66 (*br.d*, *J* = 9, CH_2), 2.82 [*d*, *J*(H-C(1), H-C(8a) = 3.8, H-C(8a)], 3.07, 3.41 (*s*, H-C(4), H-C(1)), 3.92 (*s*, CH_3O-C(6)), 3.93 (*d*, *J*(CH_3O-C(7),^{13}C(7)) = 3.1 ,CH_3O-C(7)), 6.02, 6.15 (*dd*, *J* = 5.6, H-C(2),H-C(3)]; ^{13}C NMR : 150.3 ^{13}C(7).

(6-^{13}C) (1α,4α,4aβ,8aβ)-(all-E)-3,7,11-Trimethyl-2,6,10-dodecatrienyl-1,4,4a,8a-tetrahydro-6,7-dimethoxy-4a-methyl-1,4-methanonaphtalene-5,8-dione (19a), was prepared by adopting a procedure described in ref. 7. *t*-BuOK (5 mg, 0.044 mmol) was partially dissolved in a solution of *t*-BuOH/toluene (60 µl, 4/1) at 0°C. A solution of **18a** (5 mg, 0.02 mmol) in *t*-BuOH/toluene (60 µl, 4/1) was then added dropwise. At the end of the addition, freshly distilled farnesyl bromide (29 mg, 0.1 mmol) in *t*-BuOH/toluene (60 µl, 4/1) was added rapidly. After stirring 20 min at 0°C, 2 ml of water was added and the mixture was extracted with hexane (3x2 ml). The organic extracts were combined and evaporated *in vacuo* to give an orange oil. The purification by HPLC on silicagel (hexane/ethyl acetate 9:1) gave 2.7 mg of **19a** (yield : 29%).^{1}H NMR : 1.49 (*s*, CH_3-C(4a)), 1.56, 1.58 (2*s*, 2 *allyl*-CH_3), 1.66 (*s*, *allyl*-CH_3), 1.88-2.11 (*m*, 4 *allyl*-CH_2), 2.42, 2.75 [*dd*, *J* = 8, 20, Ha-C(1'), Hb-C(1')], 3.0, 3.08 (*br.s*, H-C(1), H-C(4)), 3.87 (*s*, CH_3O-C(7)), 3.89(*d*, *J*(CH_3O-C(6),^{13}C(6) = 3.5, CH_3O-C(6)], 5.03-5.13 (*m*, 3 *allyl*-H), 6.05 (*br.s*, H-C(2), H-C(3)); ^{13}C NMR : 150.3 ^{13}C(6). (for **19** see ref.19).

(7-^{13}C) (1α,4α,4aβ,8aβ)-(all-E)-3,7,11-Trimethyl-2,6,10-dodecatrienyl-1,4,4a,8a-tetrahydro-6,7-dimethoxy-4a-methyl-1,4-methanonaphtalene-5,8-dione (19b), was prepared by the same procedure as described above. Starting from 12.5 mg of **18b**, 4.8 mg of **19b** was obtained (yield : 23 %).^{1}H NMR : 1.49 (*s*, CH_3-C(4a)), 1.58, 1.56 (2*s*, 2 *allyl*-CH_3), 1.66 (*s*, *allyl*-CH_3), 1.88-2.11 (*m*, 4 *allyl*-CH_2), 2.42, 2.75 [*dd*, *J* = 8, 20, Ha-C(1'), Hb-C(1')], 3.0, 3.08 (*br.s*, H-C(1), H-C(4)), 3.87 (*d*, *J*(CH_3O-C(7),^{13}C(7) = 3.5, CH_3O-C(7)], 3.89 (*s*, CH_3O-C(6)), 5.03-5.13 (*m*, 3 *allyl*-H), 6.05 (*br.s*, H-C(2), H-C(3)). ^{13}C NMR : 150.3 ^{13}C(7).

(5-^{13}C) 2-(all-E)-3,7,11-Trimethyl-2,6,10-dodecatrienyl-5,6-dimethoxy-3-methyl-2,5-cyclohexadien-1,4-dione ((5-^{13}C) ubiquinone-3) (20a). **19a** (2.5 mg, 0.0055 mmol) was dissolved in toluene (2 ml) and the solution was refluxed for 20 min. The solvent was evaporated *in vacuo* and an orange oil obtained and purified by HPLC on silicagel (hexane/ethyl acetate 9:1) to give 1.8 mg of **20a** (yield : 84%).^{1}H NMR : 1.56, 1.57 (2*s*, 2 *allyl*-CH_3), 1.65, 1.71 (2*s*, 2 *allyl*-CH_3), 1.95-2.06 (*m*, 4 allyl-CH_2), 2.0 (*s*, CH_3-C(3)), 3.15 [*d*, *J* = 6.8, CH_2-C(1')], 3.93 [*d*, *J*(CH_3O-C(5), ^{13}C(5)) = 3.4, CH_3O-C(5)], 3.95 (*s*, CH_3O-C(6)), 4.91 (*t*, *J*(H-C(2'),H(C1')) = 6.7, H-C(2'))], 5-5.1 (*m*, H-C(5'), H-C(8')];.^{13}C NMR : 144.7 ^{13}C(5); HREIMS : $C_{24}H_{34}O_4$: Calcd. 387.2488; Found : 387.2511; EI-MS : isotopic enrichment : 98±2%. (for **20** see ref.20).

(6-^{13}C) 2-(all-E)-3,7,11-Trimethyl-2,6,10-dodecatrienyl-5,6-dimethoxy-3-methyl-2,5-cyclohexadien-1,4-dione ((6-^{13}C) ubiquinone-3) (20b).was prepared by the same procedure as described above. Starting from 4.5 mg of **19b**, 3.2 mg of **20b** was obtained (yield : 83%).^{1}H NMR : 1.56, 1.57 (2*s*, 2 *ally*-CH_3) 1.65, 1.71 (2*s*, 2 *allyl*-CH_3), 1.95-2.06 (*m*, 4 *allyl*-CH_2), 2.0 (*s*, CH_3-C(3)), 3.15 [*d*, *J* = 6.8, CH_2-C(1')], 3.93 (*s*, CH_3O-C(5)), 3.95 (*d*, *J*(CH_3O-C(6),^{13}C(6) = 4, CH_3O-C(6)], 4.91 (*t*, *J*(H-C(2'),H(C1')) = 6.7, H-C(2'))], 5-5.1 (*m*, H-C(5'), H-C(8')]; ^{13}C NMR : 144.8 ^{13}C(6); HREIMS : $C_{24}H_{34}O_4$: Calcd. 387.2488; Found : 387.2485. EI-MS : isotopic enrichment: 98 ± 2%.

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14. 150 mg of the mixture of **6** and **7** was reduced with $Na_2S_2O_4$ to the corresponding hydroquinones which were separated by HPLC to afford 36 mg of **14** and 40 mg of **15** (yield: 50%). **14**: M.p. 112.5-113°C; UV (MeOH) 299 (3,21); IR (KBr) 3451*d*, 1468, 1407, 1311, 1195, 1034, 1005, 845, 826;^1H NMR: 2.24 (*s*, CH_3), 5.0 (*br.s*, OH-C(1), OH-C(4)), 6.81 (*s*, H-C(6)); ^{13}C NMR: 16.3 CH_3, 109.2 C(3), 116.9 C(6), 125.0 C(5), 145.2 (C(1),C(4),C(2)); EI-MS: 238 (100, $M^{+\cdot}$), 203 (53, $[M\text{-}Cl]^+$), 157 (89, $[M\text{-}Br]^+$),121,128,93,87; Anal. calcd. for $C_7H_6BrClO_2$ (237.46): C, 35.41; H, 2.52; Br, 33.65; Cl, 14.93; Found: C, 35.29; H, 2.69; Br, 33.83; Cl, 14.50. **15**: M.p. 112.4-113°C. UV (MeOH) 300 (3,52); IR (KBr) 3401*d*, 1455, 1404, 1334, 1200, 1157, 1019d, 851, 832; ^1H NMR: 2.22 (*s*, CH_3), 5.25 (*br.s*, OH-C(1,4)), 6.79 (*s*, H-C(6)); ^{13}C NMR: 16.0 CH_3, 106.2 C(2), 116.0 C(6), 125.9 C(5), 144.2 (C(1), C(4), C(3)); EI-MS: 238 (100,M^+), 201 (52, $[M\text{-}Cl]^+$), 157 (77, $[M\text{-}Br]^+$), 131, 121, 93, 87; Anal. calc. for $C_7H_6BrClO_2$ (237.46): C, 35.41; H, 2.52; Br, 33.65; Cl, 14.93; Found: C, 35.36, H, 2.62; Br, 33.07; Cl, 15.73.
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16. Each hydroquinone **14** or **15** was oxidized to regenerate the corresponding quinone **6** or **7** in quantitative yield. **6**: M.p. 105.6-106°C; UV (MeOH) 267 (4,42); IR (KBr): 2925*m*, 1626, 1560, 1262, 1130, 992, 679, 620; ^{1}H NMR: 2.15 [*d*, *J*(CH_3, H-C(6)) = 1.6, CH_3], 6.78 (*s*, H-C(6)); ^{13}C NMR: 16.3 CH_3, 132.4 C(6), 135.4 C(2), 135.5 C(3), 146.0 C(5), 176.7 C(4), 177.2 C(1); EI-MS: 236 (55, $M^{+\cdot}$), 208 (63, $[M\text{-}CO]^{+\cdot}$), 155 (80, $[M\text{-}Br]^{+\cdot}$),127 (89), 87 (100); Anal. calcd. for $C_7H_4BrClO_2$ (235.47): C, 35.71; H, 1.71; Br, 33.93; Cl, 15.06; Found: C, 35.86; H, 1.91; Br, 33.23; Cl, 14.09. **7**: M.p. 105.5-106°C; UV (MeOH) 268 (4,10); IR (KBr): 2919*m*, 1660*m*, 1570, 1261, 1132, 968, 889, 850, 673; EI-MS: 236 (80, M^+), 208 (38, $[M\text{-}CO]^+$), 155 (71, $[M\text{-}Cl]^+$), 127 (75), 99(76), 87 (100);^{1}H NMR: 2.15 (*d*, *J*(H-C(6), CH_3) = 1.6), CH_3) 6.78 (*s*, H-C(6)); ^{13}C NMR: 15.9 CH_3, 132.5 C(6), 135.4 C(2), C(3), 145.7 C(5), 176.6 C(1), 177.2 C(4); Anal. calcd. for $C_7H_4BrClO_2$ (235.47): C, 35.71; H, 1.71; Br, 33.93; Cl, 15.06; Found: C, 35.46; H, 1.85; Br, 30.33; Cl, 14.09.

17. Both quinones **6** and **7** were separately submitted to a Diels Alder reaction with cyclopentadiene to afford the adducts **16** and **17**. Starting from 50 mg of **6**, 42 mg of **16** was obtained (yield: 73%). **16**: M.p. 120.7-121°C; UV (MeOH) 275 (3,9); IR (KBr) 2977, 1766, 1684, 1554, 1452, 1229, 1116, 892, 683; EI-MS: 302 (31, $M^{+\cdot}$), 284 $[M\text{-}CO]^{+\cdot}$, 221 $[M\text{-}Br]^+$;^{1}H NMR : 1.53 (*s*, CH_3), 1.58,.172 (*d*, *J* = 9.4, CH_2), 3.02 [*d*, *J*(HC-(8a), H-C(1)) = 3.7, H-C(8a)], 3.15, 3.48 (*br.s*, H-C(1), H-C(4)), 6.02, 6.14 [*dd*, *J* = 6.3, H-C(2), H-C(3)]; ^{13}C NMR: 26.5 CH_3, 46.2 CH_2, 49.4 C(1), 54.1 C(4), 57.0 C(8a), 134.6 C(2), 138.0 C(3), 142.4 C(6), 192.2 C(5), 188.9 C(8). Anal. calcd. for $C_{12}H_{10}BrClO_2$ (301.45): C, 47.79; H, 3.34; Br, 26.5; Cl, 11.76; Found: C, 47.91; H, 3.41; Br, 27.57; Cl, 11.63. Starting from 45 mg of **7**, 36 mg of **17** was obtained (yield: 70 %).**17**: M.p.121-121.8°C. UV (MeOH) 274 (3,91). IR (KBr) 2922, 1684, 1554, 1454, 1230, 1123, 896, 686.EI-MS: 302 (59, M^+), 237 (48, $[M\text{-}CO\text{-}Cl]^+$), 221 $[M\text{-}Br]^+$, 193, 129, 87. ^{1}H NMR : same spectra as **16**. ^{13}C NMR: 26.3 CH_3, 46.2 CH_2, 49.5 C(1), 54.1 C(4), 57.0 C(8a), 134.7 C(2), 137.8 C(3), 141.9 C(6), 192.2 C(8), 188.9 C(8); Anal. calcd. for $C_{12}H_{10}BrClO_2$ (301.45): C, 47.79; H, 3.34; Br, 26.5; Cl, 11.76; Found: C, 48.98; H, 3.63; Br, 27.87; Cl, 11.63.

18. The substitution of the halogen atoms of both adducts **16** and **17** by methoxy groups led to the same intermediate (**18**) described by Rüttiman and Lorenz.

19. The alkylation with farnesyl bromide was achieved under the same conditions described for the corresponding labelled compounds to afford **19**: ^{1}H NMR : 1.49 (*s*, CH_3-C(4a)), 1.56, 1.58 (2*s*, 2 *allyl*-CH_3), 1.66 (*s*, *allyl*-CH_3), 1.88-2.11 (*m*, 4 *allyl*-CH_2), 2.42, 2.75 [*dd*, *J* = 8, 20, Ha-C(1'), Hb-C(1')], 3.0, 3.08 (*br. s*, H-C(1), H-C(4)), 3.87 (*s*, CH_3O-C(7)), 3.89 (s, CH_3O-C(6)), 5.03-5.13 (*m*, 3 *allyl*-H), 6.05 (*br.s*, H-C(2), H-C(3)).

20. The retro-Diels Alder reaction was carried out under the same conditions described in the experimental part for the corresponding labelled compounds to afford **20**: UV (EtOH) 274; IR (KBr) 1650, 1611, 1204, 1152, 1101; ^{1}H NMR : 1.56, 1.57 (2*s*, 2 CH_3-allyl), 1.65, 1.71 (2*s*, 2 *allyl*-CH_3), 1.95-2.06 (*m*, 4 *allyl*-CH_2), 2.0 (*s*, CH_3-C(3)), 3.15 [*d*, *J* = 6.8, CH_2-C(1')], 3.95 (*s*, CH_3O-C(5)), 3.96 (*s*, CH_3O-C(6)), 4.91 (*t*, *J*(H-C(2'),H(C1')) = 6.7, H-C(2'))], 5-5.1 (*m*, H-C(5'), H-C(8')]; ^{13}C NMR: 11.6 (CH_3), 16 (CH_3-C(4',9')), 17.3 C(13'), 39.6 C(8',4',12'), 61 (2 CH_3O), 119 C(2'), 124 C(6',10'), 131.5 C(7',11'), 135.6 C(3'), 137.9 C(6), 139.2 C(5), 144.9 C(2,3), 184.6 C(1), 185.4 C(4); IC/NH_3: 404 $(M+NH_4)^+$), 387 $([M+1]^+)$.

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