Synthesis and spectroscopic characterization of 5-13C and 6-13C-ubiquinone-10 for studies of bacterial photosynthetic reaction centers

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Synthesis and Spectroscopic Characterization of [5-^{13}C]- and [6-^{13}C]-Ubiquinone-10 for Studies of Bacterial Photosynthetic Reaction Centers

Rutger B. Boers,[a] Peter Gast,[b] Arnold J. Hoff,[b] Huub J. M. de Groot,[a] and Johan Lugtenburg*[a]

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This paper presents the synthesis and characterization by mass spectrometry and NMR spectroscopy of [2-^{13}C]- and [3-^{13}C]ubiquinone-0 and of [5-^{13}C]- and [6-^{13}C]ubiquinone-10. A scheme based on the synthetic approach to [5-^{13}C]ubiquinone-10 has been worked out for the synthesis of ubiquinones ^{13}C-labeled at any individual position and at every combination of positions in the quinone ring. The [5-^{13}C]- and [6-^{13}C]ubiquinone-10 isotopomers were incorporated into the Q_A-site of the photosynthetic reaction center of *Rhodobacter sphaeroides* R-26. Magic angle spinning NMR subsequently revealed an unperturbed 6-position, while the signal of the 5-position was absent. These results corroborate the recently reported detection of an asymmetric binding of Q_A with a dynamic perturbation involving the 4-carbonyl functionality.

Introduction

Ubiquinone-10 (2-decaprenyl-5,6-dimethoxy-3-methylbenzoquinone, see Figure 1; decaprenyl = 3,7,11,15,19,23,27,31,35,39-decamethyl-2,6,10,14,18,22,26,30,34,38-tetracontadecaenyl) plays an important role in redox chemistry and electron transport in the living world. It is an essential cofactor in photosynthesis and in respiration.

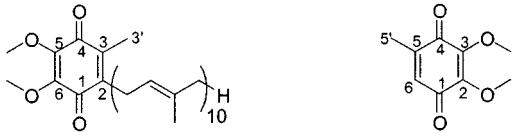

Figure 1. Structure and IUPAC numbering of ubiquinone-10 (left) and ubiquinone-0 (right); note the difference in the numbering in the quinone ring

The photosynthetic reaction center membrane protein complex of *Rhodobacter sphaeroides* R-26 makes use of two ubiquinone-10 cofactors for the conversion of light energy into chemical energy.[1] These are located in their respective Q_A- and Q_B-sites at the cytoplasmic side of the reaction center complex. They are part of two cofactor branches (A, B) comprising two bacteriochlorophyll units forming the special pair, two accessory bacteriochlorophyll units, two bacteriopheophytin units, the two ubiquinone units, and an Fe^{2+} ion. Despite near twofold symmetry in the A and B branch, the two ubiquinone units have different functions. The ubiquinone in the Q_A-site acts as a one-electron gate only and is tightly bound to the protein. It takes up an electron derived from the photoexcited special pair, to form a Q_A-radical anion species. This electron is subsequently transferred through the Fe^{2+} ion to the ubiquinone at the Q_B-site. In a second photochemically driven step, the Q_B-ubiquinone acquires another electron and in the process takes up two protons from the cytoplasm. It then leaves the reaction center as ubiquinol and is replaced by a new ubiquinone from the quinone pool in the membrane.[1]

Thus, the photon energy is converted into chemical energy by the reduction of ubiquinone into ubiquinol and, at the same time, the production of a proton gradient across the plasma membrane. This proton gradient drives the conversion of ADP into ATP, which is the universal energy carrier in living organisms.

Through the use of 1-, 2-, 3-, 3′-, and 4-^{13}C-labeled ubiquinone-10 and ($^{13}CH_3O)_2$-ubiquinone-10, obtained by total synthesis,[2] a strongly asymmetric binding of Q_A was discovered. The MAS ^{13}C NMR signal intensity of the 4-carbonyl carbon atom showed a pronounced temperature dependence, which provided the first MAS NMR evidence for dynamic behavior by Q_A.[3] In contrast, the NMR responses for the 1-, 2-, 3-, and 3′-^{13}C-labeled positions indicated that this part of the ubiquinone is essentially immobile in the binding pocket.

With the aid of FTIR spectroscopy, a dramatic bond order reduction of ca. 10% was found for the 4-carbonyl bond,[4,5] which provides additional evidence for perturbation of this side of the Q_A-quinone by the protein.[6] In the charge-separated $P^{\cdot+}Q_A^{\cdot-}$ state, in contrast, the reduced Q_A forms its strongest hydrogen bond at the 4-carbonyl posi-

[a] Leiden Institute of Chemistry, Gorlaeus Laboratory, Leiden University,
P. O. Box 9502, 2300 RA Leiden, The Netherlands
[b] Department of Biophysics, Huygens Laboratory,
P. O. Box 9504, 2300 RA Leiden, The Netherlands

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tion, while the 1-carbonyl group is thought to be weakly bonded.[4,5,7] EPR experiments have confirmed the mobility detected by MAS NMR. In the reduced form there is librational motion around the axis through the two carbonyl carbon atoms.[8] Evidence for mobility of the Q_A-plastoquinone in the analogous photosystem II reaction centers in green plants has also been found.[9]

These results provide convergent evidence for dynamic behavior by the Q_A-ubiquinone, which may be essential for the special function of this Q_A-quinone as a one-electron gate in the charge separation process. For instance, the different dynamics in the ground state and the radical anion species may play a functional role as a molecular switch, slowing down the unwanted recombination of the charge-separated state.

It is therefore essential to examine the functional mechanisms involving the ubiquinone in the Q_A-site and to determine the as yet uninvestigated interactions of the 5- and 6-positions in the Q_A-ubiquinone with the surrounding protein by MAS NMR, EPR, and FTIR, in order to establish whether these two positions, similarly to the 4-carbonyl moiety, also display some special dynamic properties in the protein.[10−12] This determination first of all requires the sufficient availability of specifically labeled [5-^{13}C]- and [6-^{13}C]ubiquinone-10.

Since it was not possible to prepare these specifically [5-^{13}C]- and [6-^{13}C]-labeled ubiquinones-10 efficiently by the synthetic schemes reported earlier,[2,13] novel schemes had to be developed. These novel schemes for the preparation of [5-^{13}C]- and [6-^{13}C]ubiquinone-10 are discussed in this paper. The new synthetic scheme for [5-^{13}C]ubiquinone-10 was extendable to enable the preparation of ubiquinones-10 ^{13}C-labeled at any single position and in all combinations of positions in the quinone ring. This should be invaluable for a full vibration analysis of the quinone ring system in the near future. Finally, the first results from MAS NMR spectroscopic investigation of the reaction centers of *Rhodobacter sphaeroides* R-26 incorporated with [5-^{13}C]- and [6-^{13}C]ubiquinone-10 into the Q_A-site are presented as the first application.

Synthesis

Most existing schemes for the synthesis of unlabeled[14−17] and deuterium-labeled[18,19] ubiquinones begin with aromatic starting materials. These cannot be modified for the ^{13}C-labeling of the ubiquinone ring at every single position and in all combinations of positions. Since there are no suitable commercially available ^{13}C-labeled aromatic compounds, the synthetic scheme has to start with ^{13}C-enriched compounds such as potassium cyanide, iodomethane, or acetic acid.

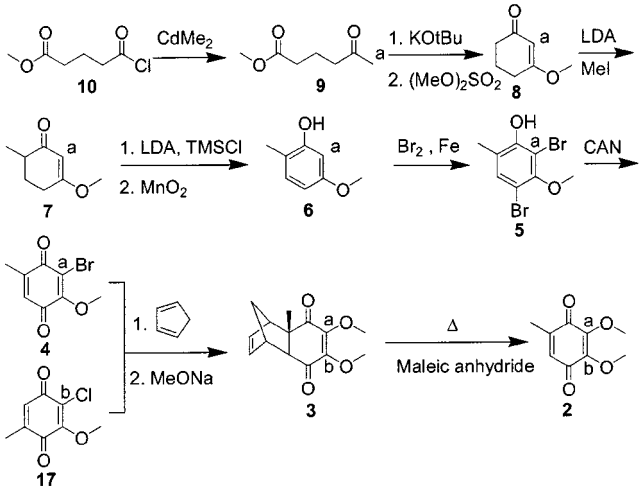
During the synthesis of ^{13}C-labeled compounds it is important to avoid synthetic steps in which dilution of the isotopic label can take place. In addition, scrambling of the label should be avoided by use of only fully regioselective reactions. To achieve this it is necessary that all reaction intermediates be asymmetric.

The first phase in this synthesis of ^{13}C-labeled ubiquinone-10 is the preparation of a labeled ubiquinone-0, starting from a suitably and correctly ^{13}C-labeled open-chain compound. We chose a ring-closure reaction to transform methyl 2-oxohexanoate into a 1,3-cyclohexadione structure[20] with all the required functionalities for the synthesis of ubiquinone-0.[21−23] The second phase was the attachment of a polyisoprenoid tail.[24−30]

For the synthesis of [5-^{13}C]- and [6-^{13}C]ubiquinones-0 we started with acid chlorides which can be converted into the corresponding methyl ketones with [$^{13}C_2$]dimethylcadmium in toluene (see Schemes 1 and 3). The [$^{13}C_2$]dimethylcadmium can be prepared by preliminary preparation of the Grignard reagent from commercially available [^{13}C]iodomethane in ether, followed by treatment with cadmium chloride. All synthetic steps were first optimized using unlabeled materials.

Synthesis of [5-^{13}C]Ubiquinone-10 (1a)

The first step in the synthesis of [5-^{13}C]ubiquinone-10 (**1a**) was the introduction of a methyl group by treatment of dimethylcadmium with methyl (4-chloroformyl)butyrate (**10**) (see Scheme 1). The resulting methyl ketone **9** underwent a ring-closure reaction with potassium *tert*-butoxide as the base[20] to form the anion of 1,3-cyclohexanedione, which was subsequently converted into the methyl enol ether **8** with dimethyl sulfate. A selective monoalkylation to give compound **7**, by treatment with LDA and iodomethane, afforded the complete carbon skeleton, with the label at the desired 2-position.



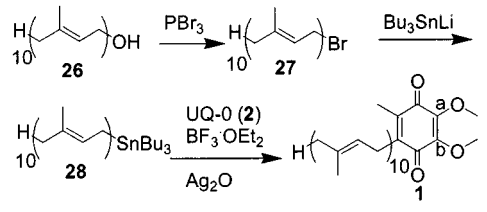
Scheme 1. Synthesis of ubiquinone-0 (a and b indicate the positions of the ^{13}C labels; **17** from Scheme 3)

Methyl enol ether **7** was aromatized to the corresponding phenol **6** by initial treatment with LDA and chlorotrimethylsilane to form a trimethylsilyl ether, followed by oxidation of this dihydrobenzene with manganese dioxide. To introduce a precursor for the methoxy functionality at the 2-position, a bromination reaction was carried out with bromine and a catalytic amount of iron. To oxidize di-

bromophenol **5** to quinone **4**, a novel treatment with cerium ammonium nitrate (CAN) was applied, as we had found that *p*-brominated phenols can be specifically oxidized to *p*-quinones in yields of around 60% with the mild CAN oxidant. Quinone **4** has all the functionalities necessary for the synthesis of ubiquinone-0 (**2**).

To exchange the bromine of quinone **4** for a methoxy group, it was necessary to perform a sequence of reactions.[2] This started with a Diels−Alder reaction with freshly distilled cyclopentadiene, and a subsequent 1,4-addition/elimination reaction with sodium methoxide to provide adduct **3**. The cyclopentadiene was then removed again by means of a retro-Diels−Alder reaction in refluxing toluene, accelerated by addition of an excess of maleic anhydride to trap the released cyclopentadiene. Ubiquinone-0 (**2**) was subsequently purified on silica.

Tributyl(decaprenyl)tin (**28**) was prepared from solanesol.[28] This 9-isoprenoid alcohol can be lengthened by one isoprene unit in a five-step reaction sequence.[2] The thus synthesized decaprenol (**26**) was transformed into the allylic bromide **27** with phosphorus tribromide (see Scheme 2), followed by treatment at low temperature with the anion of tributyltin hydride.[29]



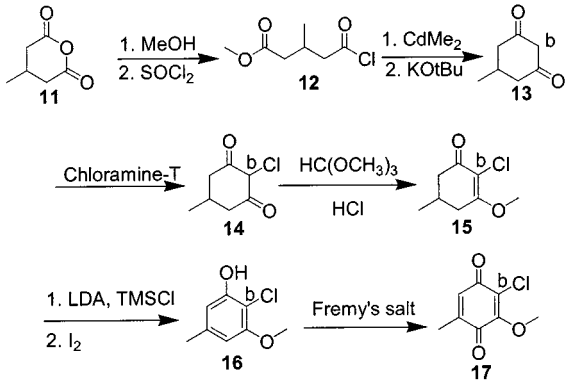
Scheme 2. Synthesis of ubiquinone-10 (a and b indicate the positions of the ^{13}C labels)

The conversion of ubiquinone-0 (**2**) into ubiquinone-10 (**1**) was carried out by means of a boron trifluoride−diethyl ether catalyzed 1,4-addition between the ubiquinone-0 (**2**) and tributyl(decaprenyl)tin (**28**).[30] Silver oxide was added to the reaction mixture to oxidize the initially formed product (ubiquinol-10) to ubiquinone-10 (**1**). Any unchanged labeled starting material could easily be recovered. The ubiquinone-10 was finally purified on silica.

The procedure described above was used to synthesize [3-^{13}C]ubiquinone-0 (**2a**, 0.21 g), starting with [$^{13}C_2$]dimethylcadmium (prepared from 10.0 g of [^{13}C]iodomethane). This was subsequently transformed into [5-^{13}C]ubiquinone-10 (**1a**, 0.37 g) in an overall yield of 2% (based on $^{13}CH_3I$). In the final purification on silica, [3-^{13}C]ubiquinone-0 (**2a**, 0.12 g) was recovered.

Synthesis of [6-^{13}C]Ubiquinone-10 (1b)

For the preparation of [6-^{13}C]ubiquinone-10 (**1b**) we were able to use the synthetic scheme developed for the synthesis of [5-^{13}C]ubiquinone-10 (**1a**), with only minor modifications. The label was now introduced with [$^{13}C_2$]dimethylcadmium by treatment with (4-chloroformyl)-3-methylbutyrate (**12**), which could be synthesized from 3-methylglutaric anhydride (**11**) by treatment with 1 equiv. of methanol followed by thionyl chloride[31] (see Scheme 3). After ring-closure of methyl ketone **13**, the chlorine atom was specifically introduced with chloramine-T.[32] The enol ether **15** was then formed with trimethyl orthoformate and a catalytic amount of HCl. This provided the complete carbon skeleton and all the functionalities to make it a suitable starting compound for the synthesis of ubiquinone-0 (**2b**).



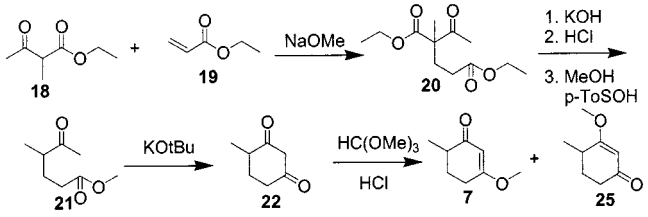
Scheme 3. Synthesis towards [6-^{13}C]ubiquinone-10 (b indicates the position of the ^{13}C-label)

The aromatization was once more performed in two steps. After formation of the silyl enol ether, it was now oxidized with iodine in a one-pot procedure.[33] Phenol **16** was subsequently oxidized by treatment with Fremy's salt[21] to quinone **17**, which was converted into ubiquinone-10 (**1**) by the same sequence as described for the [5-^{13}C]ubiquinone-10 (see Schemes 1 and 2).

The synthesis described above was used to synthesize [2-^{13}C]ubiquinone-0 (**2b**, 0.43 g), starting with [$^{13}C_2$]dimethylcadmium (made from 10.0 g of [^{13}C]iodomethane). This was subsequently transformed into [6-^{13}C]ubiquinone-10 (**1b**, 0.49 g) in an overall yield of 8% (based on $^{13}CH_3I$). After final purification on silica, [2-^{13}C]ubiquinone-0 (**2b**, 0.23 g) was recovered.

Synthesis of Specifically Labeled Ubiquinones

We realized that 3-methoxy-6-methylcyclohex-2-enone **7** (see Scheme 1) was the key molecule for developing a synthetic scheme to synthesize ubiquinones ^{13}C-labeled in all single positions and in all combinations of positions in their ring systems. It is nonsymmetric, has the complete carbon skeleton, and has all the needed functionalities present. The synthesis of this hexene **7** is based on a 1,4-addition between ethyl 2-methylacetoacetate (**18**) and ethyl acrylate (**19**) (see Scheme 4).



Scheme 4. Synthesis of 4-methyl-1,3-cyclohexanedione

Ester **18** can be prepared from ethyl bromoacetate and acetonitrile by a Blaise reaction,[34] followed by subsequent monoalkylation with iodomethane[35] in high yield. Ethyl acrylate (**19**) has been prepared from ethyl bromoacetate and formaldehyde.[36] Since ethyl bromoacetate, iodomethane, and formaldehyde are all commercially available in all ^{13}C-enriched forms, all single and all combinations of ^{13}C-labeled positions of our two starting materials **18** and **19** are accessible.

These two starting materials underwent a 1,4-addition with a catalytic amount of sodium in methanol. The produced diester **20** was converted into the monoacid in one-pot fashion by saponification and decarboxylation. After subsequent reesterification in methanol, by use of a catalytic amount of *p*-toluenesulfonic acid, methyl 4-methyl-5-oxohexanoate (**21**) underwent the ring-closure reaction to give the cyclohexanedione **22**. Subsequent synthesis of the enol ethers **7** and **25**, which could be separated on silica in relative quantities of 3:2, brought us back to the synthetic route to [5-^{13}C]ubiquinone-10 (see Scheme 1). The overall yield of enol ether **7** was 13% [from ethyl 2-methylacetoacetate (**18**)]. To increase this yield, enol ether **25** could be subjected to transetherification to provide enol ether **7**.

Spectroscopic Characterization

Mass Spectrometry

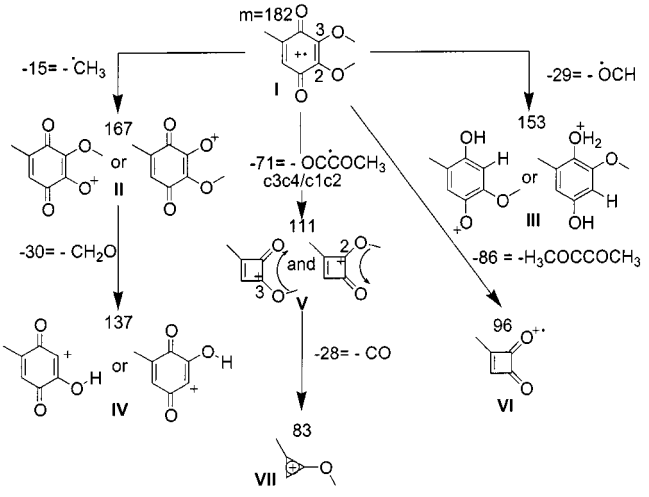
To confirm the identity and the degree of ^{13}C enrichment of [2-^{13}C]- and [3-^{13}C]ubiquinone-0 and [5-^{13}C]- and [6-^{13}C]ubiquinone-10, mass spectrometry was performed. The exact masses of these four compounds, obtained by double focus mass spectrometry, were the same (within experimental error) as the calculated values. This confirmed their molecular formulas (see Table 1).

To calculate the degree of ^{13}C incorporation, a comparison was made between the pattern of signals around the M^+ signal of natural abundance with the pattern of the synthesized labeled compounds, which should be shifted upwards by one mass unit.[37] All incorporations found in this way were close to the expected value of 99% (see Table 1). The value for [2-^{13}C]ubiquinone-0 (**2b**) was found to be slightly lower. However, we can safely assume that the degree of incorporation in **2b** was just as high as that in **1b**, since [6-^{13}C]ubiquinone-10 (**1b**; 99.6% incorporation) had been synthesized from [2-^{13}C]ubiquinone-0 (**2b**; 94.7% incorporation). The lower value was probably due to additional reduction of the sample in the MS apparatus.[43,44] These high incorporation figures confirm that no dilution of the label had taken place during the synthesis of labeled ubiquinone-10.

Examination of the mass spectroscopic fragmentation pattern of [2-^{13}C]- and [3-^{13}C]ubiquinone-0 showed some new insight in its fragmentation. Together with the specifically ^{13}C-labeled ubiquinones-0 from our earlier work,[2,38] the fragmentation to fragments of m/z = 83/84 could be worked out unequivocally (see Table 2 and Scheme 5).

Table 2. Fragments (m/z) from unlabeled [2-^{13}C]- and [3-^{13}C]ubiquinone-0 and of all other single labeled ubiquinones-0

Label position	Fragment number						
I	II	III	IV	V	VI	VII	
unlabeled	182	167	153	137	111	96	83
1	183	168	154	138	111/112	97	83/84
2	183	168	154	138	111/112	96	83
3	183	168	154	138	111/112	96	83
4	183	168	154	138	111/112	97	83/84
5	183	168	154	138	112	97	84
6	183	168	154	138	112	97	84
5′	183	168	154	138	112	97	84
($^{13}CH_3O)_2$	184	168	154	137	112	96	84



Scheme 5. Mass spectroscopic fragmentation of ubiquinone-0

Table 1. Elemental compositions of the ubiquinones, their experimental and calculated exact masses and ^{13}C incorporation

Compound	Mol. formula	Exact mass (u)		^{13}C-label incorporation
		found	calculated	
ubiquinone-0 (**2**)	$^{12}C_9H_{10}O_4$	182.0575	182.0579	–
[2-^{13}C]ubiquinone-0 (**2b**)	$^{13}C_1{}^{12}C_8H_{10}O_4$	183.0626	183.0621	94.7%
[3-^{13}C]ubiquinone-0 (**2a**)	$^{13}C_1{}^{12}C_8H_{10}O_4$	183.0637	183.0621	98.5%
ubiquinone-10 (**1**)	$^{12}C_{59}H_{90}O_4$	862.6863	862.6839	–
[5-^{13}C]ubiquinone-10 (**1a**)	$^{13}C_1{}^{12}C_{58}H_{90}O_4$	863.6914	863.6881	99.6%
[6-^{13}C]ubiquinone-10 (**1b**)	$^{13}C_1{}^{12}C_{58}H_{90}O_4$	863.6871	863.6881	98.8%

The M^+ signal common to the two compounds can be found at *m*/*z* = 183 (**I**), whereas the unlabeled quinone is found at 182 (**I**) (see Table 2). Both [2-^{13}C]- and [3-^{13}C]-ubiquinone-0 show their first two fragments (see Scheme 5) at *m*/*z* = 168 (**II**) and 154 (**III**), still containing the label. These fragments have lost either a methyl group or a formyl group from one of the methoxy groups. The next fragment **IV** at *m*/*z* = 138 also contains the ^{13}C label for both labeled ubiquinones-0. It can be formed from fragment **II** by subsequent loss of a formaldehyde molecule from the remaining methoxy group. For the fragments **V** of both [2-^{13}C]- and [3-^{13}C]ubiquinone-0 a labeled and an unlabeled signal are simultaneous found at *m*/*z* = 111 and 112, due to the loss either of the C^1C^2 carbon atoms or of the C^4C^3 carbon atoms in an $O{=}C{=}C{=}OCH_3$ molecule.[39] Fragment **VI** at *m*/*z* = 96 no longer contains a label. This fragment can therefore only be attributed to the loss of an $H_3COCCOCH_3$ molecule, containing the C^2C^3 carbon atoms. The last fragment **VII** at *m*/*z* = 83 also does not contain the C^2C^3 carbon atoms. Earlier experiments[38] had revealed that it still contains the carbonyl carbon atom and the methoxy carbon atom from fragment **V** and so must be formed by a methyl shift from the methoxy group to the carbonyl oxygen atom, followed by expulsion of the newly formed carbonyl group. The result is the cyclopropenyl cation **VII**.

The mass spectra of [5-^{13}C]- and [6-^{13}C]ubiquinones-10 are governed by two relatively stable fragments. A bicyclic oxonium ion $^{13}C^{12}C_{12}H_{15}O_4^+$ at *m*/*z* = 236 and a $^{13}C^{12}C_9H_{13}O_4^+$ fragment at *m*/*z* = 198 are observed (see Figure 2), both of which still contain the labels. The fragmentation pattern of ubiquinone-10 clearly differs from that of ubiquinone-0.

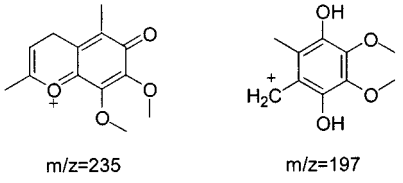


Figure 2. Structures of two stable fragments occurring in the mass spectrum of ubiquinone-10

^{1}H NMR Spectroscopy

To confirm the structure of the synthesized ubiquinones and the position of the ^{13}C labels, 300 MHz ^{1}H NMR spectra of [2-^{13}C]- and [3-^{13}C]ubiquinone-0 and 600 MHz ^{1}H NMR spectra of [5-^{13}C]- and [6-^{13}C]ubiquinone-10 were recorded. All spectra are identical to the spectra of ubiquinone-0 and ubiquinone-10 reported in the literature,[15] except for the additional couplings due to the ^{13}C isotopes. This confirms the structures of our synthesized products.

The proton spectra of the [2-^{13}C]- and [3-^{13}C]ubiquinones-0, for example, show additional coupling for one of the methoxy groups. Because it is known that three-bond coupling constants are larger than four-bond coupling constants,[40] the $^3J_{C-H}$ coupling constants (of 3.4 Hz and 3.5 Hz) can only be due to coupling between the ^{13}C label and the protons of the methoxy group directly attached to this label. This allows the signals from the two methoxy groups to be assigned unequivocally. The signals at δ = 4.02 and 4.00 can be assigned to the methoxy groups attached to C^2 and to C^3, respectively.

In the proton spectra of the two ^{13}C-labeled ubiquinones-10, additional $^3J_{C-H}$ coupling (of 3.5 Hz) with the closest methoxy group signal is observed (see Figure 3). By the same reasoning, the signals at δ = 3.98 and 3.99 can be assigned to the methoxy groups at C^5 and C^6, respectively.

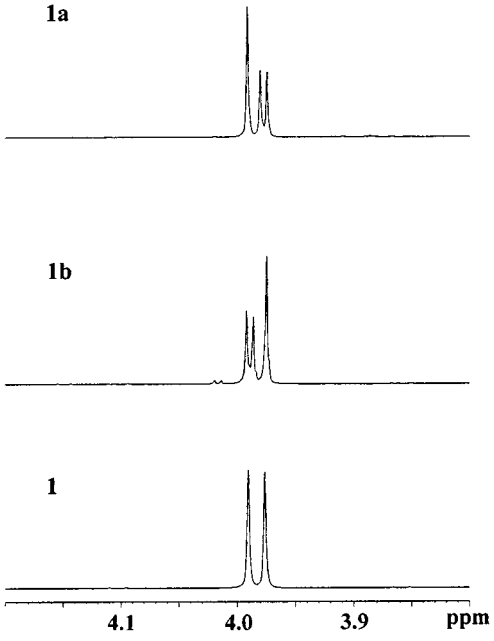


Figure 3. 600 MHz ^{1}H NMR spectra of the methoxy signals in [5-^{13}C]- (**1a**), [6-^{13}C]- (**1b**), and natural-abundance (**1**) ubiquinone-10

^{13}C NMR Spectroscopy

To verify the structures of the synthesized ubiquinones and the positions of the ^{13}C labels, we recorded ^{1}H noise-decoupled 75 MHz ^{13}C NMR spectra of [2-^{13}C]- and [3-^{13}C]ubiquinone-0 and ^{1}H noise-decoupled 150 MHz ^{13}C NMR spectra of [5-^{13}C]- and [6-^{13}C]ubiquinone-10. The spectra of the four labeled ubiquinones all show only one strong signal, at δ = 144.2 and 144.3 for the [5-^{13}C]- and [6-^{13}C]ubiquinone-10, respectively and at δ = 144.7 and 144.9 for the [2-^{13}C]- and [3-^{13}C]ubiquinone-0, respectively.

Table 3. ^{13}C-^{13}C coupling constants [Hz] in ubiquinone-0

Carbon atom	2	3	4	5	6	5′-CH_3	2-OCH_3	3-OCH_3
1	58.4	9.8	< 1.5	8.2	54.6	6.3		
2		×	9.9	< 1.5	17.3	< 1.5	8.0	2.8
3			59.7	16.7	3.0	1.7	2.6	7.8
4				49.9	9.7	< 1.5		
5					64.5	44.7		
6						< 1.5		

Table 4. ^{13}C-^{13}C coupling constants [Hz] in ubiquinone-10

Carbon atom	1	2	3	4	5	6	3′-CH_3	5′-OCH_3	6′-OCH_3
1		51.9	2.8	3.9	9.9	58.9	4.4		
2			73.0	2.4	3.0	15.9	< 1.5		
3				51.5	16.2	3.0	45.5		
4					59.1	9.9	1.5		
5						×	1.6	2.02	ca. 1.5
6							< 1.5	ca. 1.5	4.45
tail, 1-CH_2	< 1.5	44.7	< 1.5	< 1.5		1.6	2.5		
tail, 2-CH		4.1							
tail, 3-C		3.4							

This proves that only one position is labeled and that no scrambling has occurred during the synthesis. The signals of the remaining natural-abundance carbon atoms of the ubiquinones became visible when spectra with a higher signal-to-noise ratio were obtained. The additional ^{13}C-^{13}C coupling constants observed in these signals were used to confirm the location of the labels (see Tables 3 and 4). In the spectrum of [3-^{13}C]ubiquinone-0, a typical one-bond coupling constant of 59.7 Hz can be found, for example, between the (labeled) carbon-3 and the 4-carbonyl carbon atom. This confirms that the ^{13}C labels are in the desired positions and provides an unambiguous assignment of these signals.

In fact, it is now possible for the first time to make an almost complete *J*-coupling assessment for ubiquinone-0 (see Table 3)[2,38] and ubiquinone-10 (see Table 4).[2,38] Only the one-bond coupling constants between carbon atoms 2 and 3 in ubiquinone-0 and carbon atoms 5 and 6 in ubiquinone-10 are missing, due to the higher-order effects found for these carbon atoms.

Solid-State ^{13}C-NMR Reaction Center Measurements

The [5-^{13}C]- and [6-^{13}C]ubiquinone-10 were incorporated into the Q_A-sites of purified *Rhodobacter sphaeroides* R-26 reaction centers by known procedures.[3,38,41] Subsequent charge-recombination measurements[41] revealed incorporation of the Q_A-site with the labeled ubiquinones-10 of around 90%. With these reconstituted samples, 100 MHz cross-polarization ^{13}C MAS NMR spectra were recorded with a Bruker MSL 400 NMR spectrometer. In addition, difference spectra between the natural abundance protein (see Figure 4, e) and these ^{13}C-incorporated reaction centers were measured.

The NMR spectroscopic data collected for the reaction center preparation with the [6-^{13}C]ubiquinone-10 show a signal at $\delta = 147.5$ for the labeled carbon atom (see Figure 4, c and d). This is a shift of 3.2 ppm compared to the value of $\delta = 144.3$ of free [6-^{13}C]ubiquinone-10. This is comparable with the relative shifts found for the other carbon signals of the ubiquinone ring (see Figure 5).[3] The signal intensity and shift are essentially independent of temperature in the range from 210 K to 250 K. For the reaction center preparation with the [5-^{13}C]ubiquinone-10, measurements with different cross polarizations and relaxation times were performed at various temperatures down to 180 K. However, no ^{13}C-label signal was detected in the MAS NMR spectrum (see Figure 4, a and b).

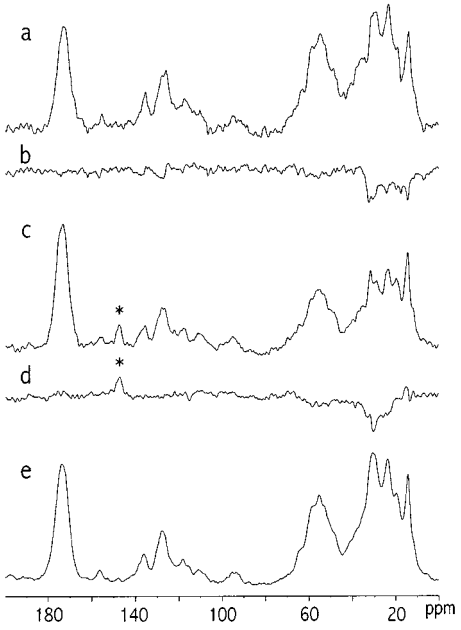


Figure 4. Solid-state NMR spectra of [5-^{13}C]- (a), [6-^{13}C]- (c), and natural-abundance (e) ubiquinone-10 incorporated into the Q_A-site of *Rhodobacter sphaeroides* R-26; the difference spectra of the two labeled spectra (a) and (b) with the natural-abundance spectrum (e) are depicted in spectra (b) and (d); the signal of the 6-carbon atom is marked by an asterisk

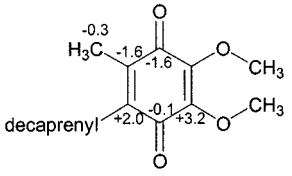


Figure 5. The chemical shift difference of ubiquinone-10 in the Q_A-site, compared with ubiquinone-10, dissolved in deuteriochloroform[3]

The difference spectrum of the [6-^{13}C]ubiquinone reconstituted reaction center protein confirms that the observed signal does not originate from the protein itself (see Figure 4, d). Similarly, the difference spectrum of the [5-^{13}C]-

ubiquinone-10 reconstituted reaction center confirms that there is no signal from the [5-^{13}C]ubiquinone-10 superimposed under one of the protein signals (see Figure 4, b). The signals at $\delta < 40$ observed in these difference spectra originate from the detergents used in purifying the protein.

Discussion

Synthesis and Characterization

The 13-step syntheses described in this paper gives access to [5-^{13}C]- and [6-^{13}C]ubiquinone-10 in relatively high overall yields of 2 and 8%, respectively, with levels of ^{13}C incorporation above 98% and without isotope scrambling. This new convergent scheme also makes it possible to label each of the two methoxy groups separately, together with the first five positions in the isoprenoid tail.[2,42]

Our earlier work on labeled ubiquinones had an overall yield of nearly 1%.[2] Its key synthetic step was a nonregioselective Diels−Alder reaction between trichloroethylene and 3-methyl-2,5-bis(trimethylsilyloxy)furan (synthesized from 2-methylglutaric anhydride). This Diels−Alder reaction is unsuitable to introduce the 5- and 6-label with satisfactory yields. A synthetic scheme has been reported for the synthesis of [5-^{13}C]- and [6-^{13}C]ubiquinone-3, where a similar reaction with a low yield of 0.01% was used.[13]

The silyl ether from ketone **15** is even more acid-sensitive then that prepared from ketone **7**, probably due to the lack of the α-methyl group. Oxidation of this silyl ether therefore had to be carried out with iodine in a one-pot fashion. This unfortunately gave a mixture of phenol **16** and 3-methoxy-5-methylphenol, levels of which could be reduced by increasing the reaction temperature and by exclusion of oxygen, which was found to oxidize only to 3-methoxy-5-methylphenol.

Treatment of the Diels−Alder adduct of quinone **4a** with methoxide produced, together with the dimethoxy Diels−Alder adduct **3a**, a small amount of the Diels−Alder adduct of cyclopentadiene and 3-methoxy-6-methyl-*para*-benzoquinone. This by-product was not found in the similar substitution reaction of the chlorinated Diels−Alder adduct made from quinone **17**.

Another improvement to our previously published work[2] is the procedure for attaching the isoprenoid tail. Diels−Alder adduct **3** was previously deprotonated by potassium *tert*-butoxide to react with an isoprenyl bromide. This anion proved to be unstable, giving only low yields (25−35%) in small-scale reactions. With the boron trifluoride−diethyl ether catalyzed 1,4-addition of tributyl-(decaprenyl)tin (**28**) to ubiquinone-0 (**2**), no ^{13}C-enriched product was lost. Unreacted starting material could be recovered and reused, to double the overall yields.

It is known that mass spectra of quinones often show strong $(M + 2)^+$ signals.[43,44] This is due to reduction of the quinone (Q) to the quinol (QH_2) in the mass spectrometer. We found that the $(M + 2)^+$ signal increases over time. By using spectra taken immediately after the sample insertion, we were able to obtain spectra without $(M + 2)^+$ signals. The ^{13}C enrichment was therefore calculated by comparison of labeled and unlabeled spectra taken immediately after insertion of the sample.

The comparison of the ^{1}H NMR spectra of ubiquinone-0 and ubiquinone-10 revealed a negligible influence of the isoprenoid tail on the chemical shift of the ubiquinone ring protons. The ring methyl group had only shifted downfield by 0.03 ppm and the methoxy signals had shifted upfield by 0.02 and 0.03 ppm after attachment of the isoprenoid tail.

In the carbon spectra, the differences were more pronounced. The carbon-2 signal had shifted upfield by 10.4 ppm upon attachment of the isoprenoid tail, whereas the signals of carbon-3 and the 3′-methyl group had shifted downfield by 5.1 and 3.5 ppm, respectively. Both carbonyl carbon signals showed small upfield shifts (< 0.4 ppm), the carbon signals at the methoxy side showed small downfield shifts (< 0.7 ppm), and the signals of the two methoxy carbon atoms had negligible shifts (< 0.1 ppm). From these shifts it can be concluded that most of the electron-donating properties of the decaprenyl tail are found at carbon-3.

Reaction Center Measurements

It is generally accepted that the binding of the Q_A-ubiquinone is asymmetric both for the neutral and for the charge-separated states, and that this asymmetric binding is induced by the protein.[3−8,45] In earlier MAS NMR studies, carbon positions 1, 2, 3, and 3′ gave clear signals.[3,38] The 4-carbonyl carbon atom gave a temperature-dependent signal, the intensity of which decreased with increasing temperature and completely disappeared above 250 K. Now, after measurement of carbon positions 5 and 6, this picture can be completed. The 6-position gave a clear signal and the 5-position was not observed, even down to 180 K. These results corroborate the detection of asymmetric binding of the Q_A-quinone with the recently reported dynamic perturbation involving the 4-carbonyl functionality.[3] The Q_A-quinone in the ground state apparently has relatively stationary carbon-1, -2, -3, -3′, and -6 and a relatively mobile carbon-4. The absence of a signal for carbon-5 is consistent with dynamic behavior parallel to that of carbon-4. The dynamic behavior of carbon-5 may be caused by a flexible methoxy group attached to this carbon atom. In conformation analysis studies, several energy-minimized metastable conformations for the two methoxy groups have been reported,[6,46,47] while different conformations of the methoxy groups have indeed been found in various reaction center crystal structure studies.[1,48−50] Carbon-6 might be restricted in its mobility by a strong hydrogen bond at the adjacent 1-carbonyl position.[51] This hydrogen bond was recently confirmed by 2D heteronuclear (^{1}H-^{13}C) dipolar correlation NMR experiments.[52]

Conclusion

The syntheses of [5-^{13}C]- and [6-^{13}C]ubiquinone-10 and of [2-^{13}C]- and [3-^{13}C]ubiquinone-0 reported here have succeeded on a 100-milligram scale in reasonably high yields

of 2% and 8%, respectively. In addition, a synthetic scheme to synthesize ubiquinones ^{13}C-labeled in all single positions and combinations of positions, starting from small, commercially available, ^{13}C-enriched materials, has been developed. NMR spectroscopy and mass spectrometry proved that the incorporations were effected without scrambling or dilution of the ^{13}C label.

The CP/MAS NMR of [6-^{13}C]ubiquinone-10 incorporated into the Q_A-site of the photosynthetic reaction center of *Rhodobacter sphaeroides* R-26 gave a clear signal, the signal intensity and shift of which were essentially independent of temperature. For the 5-labeled position, no signal could be found at temperatures down to 180 K. These findings are in line with mobility involving the carbon-4 and -5 side of the ubiquinone ring in the Q_A-site.

Experimental Section

Ether refers to diethyl ether, THF refers to tetrahydrofuran, and PE refers to low-boiling (40−60 °C) petroleum ether. Silica gel used for column chromatography was of 40−63 μm grade. All reactions were performed in flame-dried glassware under dry nitrogen or argon and with dry distilled solvents except when stated otherwise. Ether and PE were distilled from P_2O_5 and stored over sodium wire. THF and diisopropylamine were dried and stored over 4 Å molecular sieves. Methanol was distilled from magnesium turnings and stored over 3 Å molecular sieves. *tert*-Butyl alcohol was distilled from CaH_2 and stored over 4-Å molecular sieves (for distillation of *tert*-butyl alcohol a flow of air was used to cool the distillation cooler). Chlorotrimethylsilane was always freshly distilled from CaH_2 prior to use. Potassium was cut under in dry PE. Mass spectrometry was performed with a Finnigan MAT 900 (electron impact, 70 eV). Most NMR spectra were recorded with a Jeol NM FX 200. The NMR spectra of ubiquinones-0 and ubiquinone-10 were recorded with a Bruker DPX 300 instrument and a Bruker DMX 600 instrument, respectively. All samples were dissolved in deuteriochloroform except when noted otherwise. For ^1H NMR spectra, either tetramethylsilane ($\delta = 0.00$) or deuteriochloroform ($\delta = 7.26$) were used as internal standards. Deuteriochloroform ($\delta = 77.0$) was used as internal standard for the ^{13}C NMR spectra. The chemical shifts (δ values) are given in ppm. In the NMR data of the ^{13}C-labeled compounds, only those proton signals with an additional $^{13}C-H$ coupling constant, the carbon signal of the ^{13}C-labeled atom, and those carbon signals with an additional $^{13}C-^{13}C$ coupling constant are given. The additional couplings found around the ^{13}C-labeled atom are omitted, since these are the same as found in the other split carbon signals. For clarity, the numbering for the carbon atoms has been chosen in such a way that every carbon atom keeps the same number throughout the syntheses and so often does not follow the IUPAC rules. The numbering of ubiquinone-0 and ubiquinone-10 does follow the IUPAC nomenclature.

Synthesis in the Preparation of [3-^{13}C]Ubiquinone-0

Methyl 5-Oxohexanoate (9): A solution of iodomethane (10.00 g, 4.39 mL, 70.5 mmol) in ether (20 mL) was added dropwise to magnesium turnings (1.60 g, 65.8 mmol) in refluxing ether (40 mL) in such a way as to maintain the ether at a gentle reflux. To make sure no magnesium was left over, reflux was continued for about 30 min (residual magnesium seriously reduces the yields of the subsequent reactions). The Grignard mixture was cooled down to 0 °C and dry $CdCl_2$ (7.79 g, 42.5 mmol) was added, followed by reflux for 45 min. The ether solvent was then evaporated under a flow of argon and the obtained gray, sandy substance was dispersed in toluene (100 mL) at room temperature, after which methyl (4-chloroformyl)butyrate (**10**; 9.01 mL, 65.2 mmol) in toluene (10 mL) was added dropwise. After 2 h of subsequent refluxing, the mixture was cooled down to room temperature, and a saturated NH_4Cl solution (30 mL) was added, followed by acidification with 1 N HCl. After separation of the water layer, which was extracted twice with ether, the combined organic layers were washed with saturated $NaHCO_3$, NH_4Cl, and NaCl solutions and dried with $MgSO_4$. After concentration under vacuum, the yellow liquid (8.86 g) was distilled under vacuum (0.3 Torr). The fraction collected at 60−70 °C yielded methyl 5-oxohexanoate (**9**; 7.77 g) as a colorless liquid, in a yield of 70% with regard to iodomethane. ^1H NMR: $\delta = 1.9$ (m, 2 H, center CH_2), 2.15 (s, 3 H, CH_3), 2.35 (t, $^3J_{H-H}$ 7.6 Hz, 2 H, 2-CH_2), 2.53 (t, $^3J_{H-H}$ 7.2 Hz, 2 H, 4-CH_2), 3.68 (s, 3 H, OCH_3). ^{13}C NMR: $\delta = 19.8$ (3-CH_2), 29.3 (CH_3), 32.6 (2-CH_2), 42.0 (4-CH_2), 51.0 (OCH_3), 173.1 (1-C=O), 207.3 (5-C=O).

Methyl [6-^{13}C]-5-Oxohexanoate (9a): The procedure described above was performed with magnesium turnings (1.58 g, 65.0 mmol), [^{13}C]iodomethane (10.00 g, 70.0 mmol), $CdCl_2$ (7.82 g, 42.7 mmol), and methyl (4-chloroformyl)butyrate (**10**; 9.01 mL, 65.2 mmol) to yield a yellow liquid (8.86 g), which was distilled under vacuum (0.3 Torr). The fraction collected at 60−70 °C gave the target compound (7.27 g) as a colorless liquid, in a yield of 72% with regard to [^{13}C]iodomethane. ^1H NMR: $\delta = 2.15$ (d, $^1J_{C-H}$ 127.4 Hz, 3 H, $^{13}CH_3$). ^{13}C NMR: $\delta = 29.7$ ($^{13}CH_3$).

3-Methoxy-2-cyclohexenone (8): Potassium (1.50 g, 38.4 mmol) was added to *tert*-butyl alcohol (250 mL). After all the potassium had reacted, methyl 5-oxohexanoate (**9**; 5.00 g, 34.7 mmol) was added and the mixture was stirred overnight. The *tert*-butyl alcohol was evaporated under vacuum and the obtained brown solid was dissolved in dichloromethane (150 mL). After addition of dimethyl sulfate (5.0 mL, 52.8 mmol), the mixture was refluxed for 6 h. After the mixture had cooled down to room temperature, water (50 mL) and concentrated ammonia (25 mL) were added and the mixture was stirred for 15 min, followed by addition of concentrated HCl (50 mL), dissolved in water (100 mL). The water layer was separated and extracted three times with dichloromethane. The combined organic layers were dried with $MgSO_4$. After concentration under vacuum, a crude yield of 4.37 g (100%) was obtained. ^1H NMR: $\delta = 2.0$ (m, 2 H, 5-CH_2), 2.34 (t, $^3J_{H-H}$ 6.9 Hz, 2 H, CH_2), 2.43 (t, $^3J_{H-H}$ 5.8 Hz, 2 H, CH_2), 3.72 (s, 3 H, OCH_3), 5.37 (s, 1 H, CH). ^{13}C NMR: $\delta = 20.5$ (5-CH_2), 28.1 (4-CH_2), 35.9 (6-CH_2), 54.9 (OCH_3), 101.3 (CH), 178.3 (CO), 198.9 (C=O).

[2-^{13}C]-3-Methoxy-2-cyclohexenone (8a): The procedure described above was performed with *tert*-butyl alcohol (360 mL), potassium (2.18 g, 55.8 mmol), methyl [6-^{13}C]-5-oxohexanoate (**9a**; 7.27 g, 50.1 mmol), dichloromethane (220 mL), and dimethyl sulfate (7.27 mL, 76.8 mmol) for the reactions, water (70 mL) and concentrated ammonia (35 mL) to destroy the dimethyl sulfate, and concentrated HCl (75 mL) in water (145 mL) for the workup procedure. After evaporation under vacuum, a crude yield of 5.76 g (94%) was obtained. ^1H NMR: $\delta = 5.37$ (d, $^1J_{C-H}$ 160.0 Hz, 1 H, ^{13}CH). ^{13}C NMR: $\delta = 102.1$ (^{13}CH).

3-Methoxy-6-methyl-2-cyclohexenone (7): At −20 °C, diisopropylamine (6.26 mL, 44.7 mmol) was dissolved in THF (65 mL), followed by addition of a butyllithium solution in hexane (1.6 M, 28.6 mL, 45.8 mmol) with a syringe. After 10 min, this mixture was cooled down to −80 °C, after which 3-methoxy-2-cyclohexenone (**8**; 4.37 g, 34.7 mmol) in THF (16 mL) was added dropwise. After

10 min this was followed by dropwise addition of iodomethane (3.06 mL, 49.2 mmol). This mixture was allowed to warm up to −15 °C over about 2 h, after which a saturated NH_4Cl solution (25 mL) was added. The water layer was separated and extracted three times with ether. The combined organic layers were dried with $MgSO_4$. After concentration under vacuum, a crude yield of 4.16 g (86%) was obtained. ^{1}H NMR: δ = 1.15 (d, $^3J_{H-H}$ 6.9 Hz, 3 H, CH_3), 1.7−2.5 (several m, 5 H, 2-CH_2 and 6-CH), 3.69 (s, 3 H, OCH_3), 5.34 (s, 1 H, 2-CH). ^{13}C NMR: δ = 15.0 (CH_3), 27.8 (5-CH_2), 28.9 (4-CH_2), 39.8 (6-CH), 55.3 (OCH_3), 101.2 (2-CH), 177.3 (CO), 201.3 (C=O).

[2-^{13}C]-3-Methoxy-6-methyl-2-cyclohexenone (7a): The procedure described above was performed with diisopropylamine (8.26 mL, 58.9 mmol) in THF (85 mL), a butyllithium solution in hexane (1.6 M, 37.7 mL, 60.3 mmol), [2-^{13}C]-3-methoxy-2-cyclohexenone (**8a**; 5.76 g, 45.4 mmol) in THF (20 mL), and iodomethane (4.03 mL, 64.7 mmol) for the reactions and saturated NH_4Cl solution (25 mL) for the workup procedure. After concentration under vacuum, a crude yield of 5.80 g (91%) was obtained. ^{1}H NMR: δ = 5.34 (d, $^1J_{C-H}$ 159.3 Hz, 1 H, ^{13}CH). ^{13}C NMR: δ = 101.5 (^{13}CH).

Trimethylsilyl Enol Ether of 3-Methoxy-6-methyl-2-cyclohexenone: At −15 °C, diisopropylamine (4.66 mL, 33.2 mmol) was dissolved in THF (43 mL), followed by addition (by syringe) of a butyllithium solution in hexane (1.6 M, 18.7 mL, 29.9 mmol). After 10 min, this was followed by dropwise addition of 3-methoxy-6-methyl-2-cyclohexenone (**7**; 4.16 g, 29.7 mmol), dissolved in THF (17 mL). After 5 min, this was followed by addition of chlorotrimethylsilane (8.31 mL, 65.5 mmol). This mixture was allowed to warm to room temperature over 1 h, after which the reaction mixture was poured into a quickly stirred saturated Na_2CO_3 solution, followed by fast separation in a separating funnel and immediate drying of the organic layer with $MgSO_4$. The water layer was quickly extracted once with ether and the organic phase was combined with the first $MgSO_4$-containing batch to dry. After concentration under vacuum, a crude yield of 7.22 g (115%) was obtained and immediately used in the subsequent MnO_2 oxidation. ^{1}H NMR: δ = 0.16 [s, 9 H, $Si(CH_3)_3$], 1.62 (s, 3 H, CH_3), 2.2 (m, 4 H, 2CH_2), 3.55 (s, 3 H, OCH_3), 4.70 (s, 1 H, CH).

Trimethylsilyl Enol Ether of [2-^{13}C]-3-Methoxy-6-methyl-2-cyclohexenone: The procedure described above was performed with diisopropylamine (6.50 mL, 46.4 mmol) in THF (60 mL), a butyllithium solution in hexane (1.6 M, 26.1 mL, 41.8 mmol), [2-^{13}C]-3-methoxy-6-methyl-2-cyclohexenone (**7a**; 5.80 g, 41.4 mmol) in THF (24 mL), and chlorotrimethylsilane (11.6 mL, 91.4 mmol). A crude yield of 8.08 g (92%) was obtained and immediately used in the subsequent MnO_2 oxidation. ^{1}H NMR: δ = 4.70 (d, $^1J_{C-H}$ 122.9 Hz, 1 H, ^{13}CH). ^{13}C NMR: δ = 94.8 (^{13}CH).

3-Methoxy-6-methylphenol (6): Active MnO_2 powder (10 g) was added to the crude trimethylsilyl enol ether of 3-methoxy-6-methyl-2-cyclohexenone (7.22 g), dissolved in undistilled (wet!) ether (30 mL). This mixture was stirred overnight and subsequently dried with $MgSO_4$ and filtered through Hyflo Super Cel. The residue was extracted thoroughly with ether. After concentration under vacuum, a crude yield of 6.26 g (121%) was obtained. ^{1}H NMR: δ = 2.10 (s, 3 H, CH_3), 3.74 (s, 3 H, OCH_3), 6.36 (d, $^4J_{H-H}$ 2.8 Hz, 1 H, 2-CH), 6.44 (dd, $^3J_{H-H}$ 8.2 Hz and $^4J_{H-H}$ 2.8 Hz, 1 H, 4-CH), 7.01 (d, $^3J_{H-H}$ 8.2 Hz, 1 H, 5-CH). ^{13}C NMR: δ = 15.2 (CH_3), 54.7 (OCH_3), 105.3 (4-CH), 105.5 (2-CH), 120.6 (C), 130.3 (5-CH), 153.7 (COH), 157.9 (CO).

[2-^{13}C]-3-Methoxy-6-methylphenol (6a): The procedure described above was performed with the crude trimethylsilyl enol ether of [2-^{13}C]-3-methoxy-6-methyl-2-cyclohexenone (8.08 g) in undistilled (wet!) ether (30 mL) and active MnO_2 (10 g) to obtain a crude yield of 6.26 g (109%). ^{1}H NMR: δ = 6.36 (dd, $^4J_{H-H}$ 2.8 Hz and $^1J_{C-H}$ 156.5 Hz, 1 H, ^{13}CH), 6.44 (ddd, $^3J_{H-H}$ 8.2, $^4J_{H-H}$ 2.8 Hz and $^3J_{C-H}$ 5.2 Hz, 1 H, 4-CH). ^{13}C NMR: δ = 105.8 (^{13}CH).

2,4-Dibromo-3-methoxy-6-methylphenol (5): Iron powder (0.22 g, 3.9 mmol) was added to 3-methoxy-6-methylphenol (**6**; 4.97 g, 36.0 mmol) dissolved in dichloromethane (110 mL), cooled to 0 °C. This was followed by dropwise addition of bromine (4.85 mL, 94.1 mmol) in dichloromethane (40 mL) in the dark. After this had stirred overnight, some saturated sodium dithionite solution was added to destroy the excess bromine and the mixture was subsequently acidified with 1 N HCl to pH = 2−3. The water layer was separated and extracted three times with dichloromethane. The combined organic layers were dried with $MgSO_4$ and the solvents were evaporated under vacuum. This resulted in a crude yield of 8.06 g [73% based on 3-methoxy-6-methyl-2-cyclohexenone (**7**)]. ^{1}H NMR: δ = 2.21 (s, 3 H, CH_3), 3.82 (s, 3 H, OCH_3), 5.7 (br. s, 1 H, OH), 7.23 (s, 1 H, CH). ^{13}C NMR: δ = 16.0 (CH_3), 60.6 (OCH_3), 106.4 (2-CBr), 106.8 (4-CBr), 131.7 (C), 132.9 (5-CH), 150.7 (COH), 151.8 (CO).

[2-^{13}C]-2,4-Dibromo-3-methoxy-6-methylphenol (5a): The procedure described above was performed with 3-methoxy-6-methylphenol (**6a**, 6.26 g, 45.0 mmol) in dichloromethane (110 mL), iron powder (0.28 g, 5.0 mmol), and bromine (4.85 mL, 94.1 mmol) in dichloromethane (40 mL) to obtain a crude yield of 8.06 g {66% based on [2-^{13}C]-3-methoxy-6-methyl-2-cyclohexenone (**7a**)}. ^{13}C NMR: δ = 106.4 (^{13}CBr).

2-Bromo-3-methoxy-6-methylparabenzoquinone (4): 2,4-Dibromo-3-methoxy-6-methylphenol (**5**; 6.46 g, 21.8 mmol) and 2,6-pyridinedicarboxylic acid (20.5 g, 123 mmol) were dissolved in a mixture of water (205 mL) and acetonitrile (205 mL). This mixture was cooled to 0 °C, after which ammonium cerium(IV) nitrate (51 g, 93.0 mmol) was added in 5-g portions in 1-min intervals. This mixture was stirred overnight at room temperature. Some ether was then added and the water layer was separated and extracted three times with ether. The combined organic layers were washed with a $NaHCO_3$ solution at pH = 9, a saturated NH_4Cl solution, and a saturated NaCl solution, followed by drying with $MgSO_4$. After concentration under vacuum, a crude yield of 4.79 g was obtained. This was introduced onto a silica column with the aid of some dichloromethane and was eluted with 15% ether/85% PE. This yielded 0.83 g of pure quinone [10% based on methyl 5-oxohexanoate (**9**)]. ^{1}H NMR: δ = 2.12 (d, $^4J_{H-H}$ 1.4 Hz, 3 H, CH_3), 4.21 (s, 3 H, OCH_3), 6.51 (q, $^4J_{H-H}$ 1.4 Hz, 1 H, CH). ^{13}C NMR: δ = 16.2 (CH_3), 61.4 (OCH_3), 117.9 (CBr), 131.1 (CH), 145.5 (C), 156.6 (CO), 180.4 (C=O), 180.9 (C=O).

[2-^{13}C]-2-Bromo-3-methoxy-6-methyl-*para*-benzoquinone (4a): The procedure described above was performed with [2-^{13}C]-2,4-dibromo-3-methoxy-6-methylphenol (**5a**; 8.06 g, 27.3 mmol), 2,6-pyridinedicarboxylic acid (25.6 g, 153 mmol), water (260 mL), acetonitrile (260 mL), and ammonium cerium nitrate (64 g, 117 mmol) to obtain a crude yield of 5.15 g. This was introduced onto a silica column with the aid of some dichloromethane and was eluted with 15% ether/85% PE to yield pure quinone [1.28 g; 11% based on methyl 5-oxohexanoate (**9a**)]. ^{13}C NMR: δ = 118.1 (^{13}CBr).

Diels−Alder Adduct of Cyclopentadiene and 2-Bromo-3-methoxy-6-methyl-*para*-benzoquinone. − 2-Bromo-3-methoxy-8aα-methyl-4a,5,8,8a-tetrahydro-5α,8α-methano-1,4-naphthoquinone: 2-Bromo-3-methoxy-6-methyl-*para*-benzoquinone (**4**; 0.83 g, 3.59 mmol) and freshly distilled cyclopentadiene (5 mL) were dissolved in dichloro-

methane (5 mL). After this mixture had been stirred for 40 h, the solvent was evaporated under vacuum and replaced by PE (10 mL). The excess cyclopentadiene was removed in a flash column with PE as eluent. The product was removed from the flash column by elution with ether. After evaporation of the solvent under vacuum, 1.06 g (100%) of pure product was obtained. ^{1}H NMR: δ = 1.53 (s, 3 H, 8aα-CH_3), 1.57 (d, $^2J_{H-H}$ 8.9 Hz, 1 H, CH_AH$_B$), 1.71 (d, $^2J_{H-H}$ 8.9 Hz, 1 H, CH$_A$$H_B$), 2.91 (d, $^3J_{H-H}$ 3.8 Hz, 1 H, 4a-CH), 3.13 (br. s, 1 H, CH), 3.44 (br. s, 1 H, CH), 4.12 (s, 3 H, OCH_3), 6.02 (dd, $^3J_{H-H}$ 2.7 Hz and 5.5 Hz, 1 H, CH=*CH*), 6.15 (dd, $^3J_{H-H}$ 2.7 Hz and 5.8 Hz, 1 H, *CH*=CH). ^{13}C NMR: δ = 26.9 (8aα-CH_3), 46.3 (CH_2), 48.7 (CH), 53.7 (CH), 57.9 (OCH_3), 60.2 (4a-C), 60.9 (4a-CH), 125.2 (CBr), 134.4 (CH=*CH*), 138.3 (*CH*=CH), 161.5 (CO), 192.2 (C=O), 194.5 (C=O).

Diels−Alder Adduct of Cyclopentadiene and [2-^{13}C]-2-Bromo-3-methoxy-6-methyl-*para*-benzoquinone: The procedure described above was performed with [2-^{13}C]-2-bromo-3-methoxy-6-methyl-*para*-benzoquinone (1.28 g, 4.30 mmol) in dichloromethane (5 mL) and freshly distilled cyclopentadiene (5 mL) to obtain 1.60 g (97%) of pure product. ^{13}C NMR: δ = 125.3 (^{13}CBr).

Synthesis of Ubiquinone-0 (note the change to ubiquinone-0 numbering)

Diels−Alder Adduct of Cyclopentadiene and Ubiquinone-0 (3). − 2,3-Dimethoxy-4aα-methyl-4a,5,8,8a-tetrahydro-5α,8α-methano-1,4-naphthoquinone: Sodium (1.80 g 78.3 mmol) was added to methanol (20 mL), with sufficient cooling. From this solution, 6.1 mL was taken and added to a solution (cooled to 0 °C) of the Diels−Alder adduct of cyclopentadiene and 2-bromo-3-methoxy-6-methyl-*para*-benzoquinone (1.06 g, 3.57 mmol) in toluene (40 mL). After 15 min of stirring at 0 °C, the mixture was allowed to warm to room temperature and stirred for another 1.75 h, after which the mixture was poured into saturated NH_4Cl solution (20 mL). The water layer was separated and extracted twice with ether. The combined organic layers were dried with $MgSO_4$ and the solvents were evaporated under vacuum, to yield 0.63 g (71%) of crude product. ^{1}H NMR: δ = 1.49 (s, 3 H, 4aα-CH_3), 1.55 (d, $^2J_{H-H}$ 8.9 Hz, 1 H, CH_AH$_B$), 1.68 (d, $^2J_{H-H}$ 8.9 Hz, 1 H, CH$_A$$H_B$), 2.84 (d, $^3J_{H-H}$ 4.1 Hz, 1 H, 8a-CH), 3.09 (br. s, 1 H, CH), 3.43 (br. s, 1 H, CH), 3.93 (s, 3 H, OCH_3), 3.95 (s, 3 H, OCH_3), 6.02 (dd, $^3J_{H-H}$ 2.8 Hz and 5.5 Hz, 1 H, *CH*=CH), 6.16 (dd, $^3J_{H-H}$ 2.8 Hz and 5.5 Hz, 1 H, CH=*CH*). ^{13}C NMR: δ = 26.3 (4aα-CH_3), 46.2 (CH_2), 48.6 (CH), 52.4 (8a-C), 53.2 (CH), 56.9 (2OCH_3), 60.4 (8a-CH), 134.3 (*CH*=CH), 137.9 (CH=*CH*), 150.3 and 150.4 (CO), 194.6 and 198.2 (C=O).

Diels−Alder Adduct of Cyclopentadiene and [3-^{13}C]Ubiquinone-0 (3a): The procedure described above was performed with the sodium methoxide solution (9.2 mL) and the Diels−Alder adduct of cyclopentadiene and [2-^{13}C]-2-bromo-3-methoxy-6-methyl-*para*-benzoquinone (1.60 g, 4.30 mmol) in toluene (40 mL), to obtain the crude product (1.26 g, 94%). Purification on a silica column with 50% ether/50% PE as eluent yielded 0.38 g (28%) of product. Unfortunately, 0.24 g (20%) of the Diels−Alder adduct of cyclopentadiene and [2-^{13}C]-3-methoxy-6-methyl-*para*-benzoquinone was also obtained. ^{1}H NMR: δ = 3.93 (d, $^3J_{C-H}$ 3.4 Hz, 3 H, ^{13}CO*C*H_3). ^{13}C NMR: δ = 150.4 (^{13}CO).

Diels−Alder Adduct of Cyclopentadiene and [2-^{13}C]Ubiquinone-0 (3b): The procedure described above was performed with the sodium methoxide solution (8 mL) and the Diels−Alder adduct of cyclopentadiene and [2-^{13}C]-2-chloro-3-methoxy-5-methyl-*para*-benzoquinone (1.34 g, 5.28 mmol) in toluene (25 mL). After 2 h of stirring at 0 °C, 1.01 g (77%) of crude product was obtained. ^{1}H NMR: δ = 2.84 (dd, $^3J_{H-H}$ 4.1 Hz and $^3J_{C-H}$ 2.1 Hz, 1 H, 8a-CH), 3.95 (d, $^3J_{C-H}$ 3.4 Hz, 3 H, ^{13}CO*C*H_3). ^{13}C NMR: δ = 150.5 (^{13}CO).

Diels−Alder Adduct of Cyclopentadiene and Ubiquinone-0 (3): Freshly distilled cyclopentadiene (10 mL) was added to ubiquinone-0 (**2**; 2.00 g, 11.0 mmol) in dichloromethane (5 mL). After 4 d of stirring, the solvent was evaporated under vacuum and replaced by PE (10 mL). The excess cyclopentadiene was removed in a flash column with PE as solvent. The product was obtained by flushing with ether and evaporation of the solvent under vacuum. The yield of pure product was 2.72 g (100%).

Ubiquinone-0 (2)

2,3-Dimethoxy-5-methyl-*para*-benzoquinone: Maleic anhydride (1.05 g, 10.7 mmol) and the Diels−Alder adduct of cyclopentadiene and 2,3-dimethoxy-5-methyl-*para*-benzoquinone (**3**) (1.75 g, 7.06 mmol) were added to toluene (35 mL) and the mixture was heated at reflux for 4 h. Water (20 mL) at pH = 9 was then added at room temperature and the mixture was stirred for 15 min. The water phase was separated and extracted three times with ether. The combined organic layers were dried with $MgSO_4$. Concentration under vacuum yielded 2.62 g of crude product. After purification on silica with 50% ether/50% PE as eluent, pure ubiquinone-0 (**2**; 1.23 g, 96%) was obtained. ^{1}H NMR (300 MHz): δ = 2.04 (d, $^4J_{H-H}$ 1.6 Hz, 3 H, CH_3), 4.00 (s, 3 H, 3′-OCH_3), 4.02 (s, 3 H, 2′-OCH_3), 6.44 (q, $^4J_{H-H}$ 1.6 Hz, 1 H, CH). ^{13}C NMR (75 MHz): δ = 15.4 (CH_3), 61.1 (2′-OCH_3), 61.2 (3′-OCH_3), 131.2 (CH), 143.9 (C), 144.7 (2-CO), 144.9 (3-CO), 183.8 (1-C=O), 184.3 (4-C=O).

[3-^{13}C]Ubiquinone-0 (2a): The procedure described above was performed with maleic anhydride (0.23 g, 2.35 mmol) in toluene (10 mL), and the Diels−Alder adduct of cyclopentadiene and [3-^{13}C]-2,3-dimethoxy-5-methyl-*para*-benzoquinone (**3a**) (0.38 g, 1.53 mmol) to obtain crude product (0.60 g). After purification on silica, pure [3-^{13}C]ubiquinone-0 (0.21 g, 75%) was obtained. ^{1}H NMR (300 MHz): δ = 4.00 (d, 3.5 Hz, 3 H, 3′-OCH_3) 6.44 (dq, $^4J_{H-H}$ 1.6 Hz and $^4J_{C-H}$ 0.6 Hz, 1 H, CH). ^{13}C NMR (75 MHz): δ = 15.4 (d, $^3J_{C-C}$ 1.7 Hz, CH_3), 61.1 (d, $^3J_{C-C}$ 2.6 Hz, 2′-OCH_3), 61.2 (d, $^2J_{C-C}$ 7.8 Hz, 3′-OCH_3), 131.2 (d, $^3J_{C-C}$ 3.0 Hz, CH), 143.9 (d, $^2J_{C-C}$ 16.7 Hz, C), no signal found for 2-CO, 144.9 (3-^{13}CO), 184.1 (d, $^2J_{C-C}$ 9.8 Hz, 1-C=O), 184.3 (d, $^1J_{C-C}$ 59.7 Hz, 4-C=O).

[2-^{13}C]Ubiquinone-0 (2b): The procedure described above was performed with maleic anhydride (0.61 g, 6.22 mmol) in toluene (25 mL) and the Diels−Alder adduct of cyclopentadiene and [2-^{13}C]-2,3-dimethoxy-5-methyl-*para*-benzoquinone (**3b**, 1.01 g, 4.06 mmol) to obtain the crude product (1.49 g). After purification on silica, pure [2-^{13}C]ubiquinone-0 (0.43 g, 65% based on the Diels−Alder adduct of cyclopentadiene and [2-^{13}C]-2-chloro-3-methoxy-5-methyl-*para*-benzoquinone) was obtained. ^{1}H NMR (300 MHz): δ = 4.02 (d, $^3J_{C-H}$ 3.4 Hz, 3 H, 2′-OCH_3), 6.44 (dq, $^4J_{H-H}$ 1.6 Hz and $^3J_{C-H}$ 6.5 Hz, 1 H, CH). ^{13}C NMR (75 MHz): δ = 15.4 (s, CH_3), 61.1 (d, $^2J_{C-C}$ 8.0 Hz, 2′-OCH_3), 61.1 (d, $^2J_{C-C}$ 2.8 Hz, 3′-OCH_3), 131.2 (d, $^2J_{C-C}$ 17.3 Hz, CH), 143.9 (d, $^3J_{C-C}$ 3.0 Hz, C), 144.7 (2-^{13}CO), no signal found for 3-CO, 184.1 (d, $^1J_{C-C}$ 58.4 Hz, 1-C=O), 184.3 (d, $^2J_{C-C}$ 9.9 Hz, 4-C=O).

Synthesis of Ubiquinone-10 (note the change to ubiquinone-10 numbering)

Decaprenyl Bromide (27). − (all-*E*)-3,7,11,15,19,23,27,31,35,39-Decamethyl-2,6,10,14,18,22,26,30,34,38-tetracontadecaenyl Bromide: Decaprenol (**26**, 2.76 g, 3.96 mmol) and pyridine (10 drops) were dissolved in ether (38 mL) and PE (24 mL). This mixture was co-

oled in an ice bath, after which phosphorus tribromide (0.32 mL, 3.37 mmol) was added dropwise. The mixture was stirred at 0 °C for 1 h, followed by 1 h at room temperature, after which the mixture was carefully poured into a separating funnel partially filled with water, ice, and PE. The water layer was separated and extracted three times with PE. The combined organic layers were carefully dried with $MgSO_4$ and the solvent was evaporated under vacuum. A crude yield of 3.03 g (100%) was obtained. ^{1}H NMR: δ = 1.60 (s, 27 H, 9 CH_3), 1.68 (s, 3 H, 40-CH_3), 1.72 (s, 3 H, 3′-CH_3), 2.01 (m, 36 H, 18 CH_2), 4.02 (d, $^3J_{H-H}$ 8.2 Hz, 2 H, CH_2Br), 5.1 (br. t, $^3J_{H-H}$ 7 Hz, 9 H, 9 CH), 5.53 (t, $^3J_{H-H}$ 8.2 Hz, 1 H, 2-CH).

Tributyl(decaprenyl)tin (28)

Tributyl[(all-*E*)-3,7,11,15,19,23,27,31,35,39-decamethyl-2,6,10,14,18,22,26,30,34,38-tetracontadecaenyl]tin: A solution of butyllithium in hexane (1.6 M, 4.53 mL, 7.25 mmol) was injected by syringe into diisopropylamine (1.16 mL, 8.28 mmol) in THF (10 mL) cooled to 0 °C. After 5 min, tributyltin hydride (1.18 mL, 4.39 mmol) was added. This mixture was stirred for 15 min and subsequently cooled to −60 °C, after which decaprenyl bromide (**27**, 2.78 g, 3.66 mmol) in THF (20 mL) was added dropwise. The mixture was allowed to warm up to −40 °C over 2 h, after which the reaction was quenched by pouring it into some saturated NH_4Cl. The water layer was separated and extracted twice with ether. The combined organic layers were washed with saturated NaCl solution and carefully dried with $MgSO_4$. After evaporation of the solvent under vacuum, a crude yield of 3.61 g (102%) was obtained. ^{1}H NMR: δ = 0.8 (m, 15 H, 3-butyl-CH_2Sn and 3-butyl-CH_3), 1.3 (m, 6 H, 3-butyl-CH_2), 1.5 (m, 8 H, 3 butyl-CH_2 and 1 CH_2Sn), 1.60 (s, 27 H, 9 CH_3), 1.68 (s, 6 H, 3′-CH_3 and *cis*-39′-CH_3), 2.00 (m, 36 H, 18 CH_2), 5.1 (br. t, $^3J_{H-H}$ 7 Hz, 9 H, 9 CH), 5.32 (t, $^3J_{H-H}$ 9.3 Hz, 1 H, 3-CH).

Ubiquinone-10 (1)

2-[(all-*E*)-3,7,11,15,19,23,27,31,35,39-Decamethyl-2,6,10,14,18,30,34,38-tetracontadecaenyl]-5,6-dimethoxy-3-methylpara-22,26,benzoquinone: Silver oxide (0.64 g, 2.76 mmol) was added to ubiquinone-0 (**2**; 0.20 g, 1.10 mmol) in dichloromethane (12 mL). This mixture was kept in the dark and cooled to −80 °C, after which boron trifluoride−diethyl ether (0.85 mL, 6.91 mmol), freshly distilled from CaH_2, was injected by syringe. Tributyl(decaprenyl)tin (**28**; 1.53 g, 1.58 mmol) in dichloromethane (10 mL) was then added dropwise. This mixture was allowed to warm up to −20 °C over 2 h and was then cooled down to −40 °C, after which saturated NaCl solution (20 mL) was added and the cooling removed. After addition of potassium fluoride (4 g), the mixture was stirred for 30 min and then filtered through Hyflo Super Cel. The water layer was separated and extracted twice with dichloromethane. The combined organic layers were dried with $MgSO_4$ and the solvents were evaporated under vacuum. A crude yield of 1.71 g was obtained. Purification was on silica with an eluent of 20% ether/80% PE until elution of ubiquinone-10, followed by 50% ether/50% PE for the ubiquinone-0. This yielded pure ubiquinone-10 (**1**; 0.21 g, 0.24 mmol, 22%) and pure ubiquinone-0 (**2**; 0.14 g, 0.77 mmol, 70%). If the product still contained some ubiquinols, then an oxidation procedure was performed with ammonium cerium nitrate as described for the oxidation to 2-bromo-3-methoxy-6-methyl-*para*-benzoquinone (**4**) prior to purification on silica. ^{1}H NMR (600 MHz): δ = 1.57 (s, 3 H, tail end *trans*-40-CH_3), 1.59 (s, 3 H, tail-CH_3), 1.60 (s, 21 H, tail 7CH_3), 1.68 (s, 3 H, tail end *cis*-39′-CH_3), 1.74 (s, 3 H, tail 3′-CH_3), 1.98 and 2.06 (m, 36 H, tail 18 CH_2), 2.01 (s, 3 H, 5′-CH_3), 3.18 (d, $^3J_{H-H}$ 7.2 Hz, 2 H, tail 1-CH_2), 3.98 (s, 3 H, 5′-OCH_3), 3.99 (s, 3 H, 6′-OCH_3), 4.93 (t, $^3J_{H-H}$ 7.2 Hz, 1 H, tail 2-CH), 5.06 (t, $^3J_{H-H}$ 6.0 Hz, 1 H, tail 6-CH), 5.11 (br. t, $^3J_{H-H}$ 6.6 Hz, 8 H, tail 8 CH). ^{13}C NMR (150 MHz): δ = 11.9 (3′-CH_3), 16.0 (m, tail 7′-, 11′-, 15′-, 19′-, 23′-, 27′-, 31′-, and 35′-CH_3), 16.3 (tail 3′-CH_3), 17.6 (tail end *cis*-39′-CH_3), 25.3 (tail 1-CH_2), 25.7 (tail end *trans*-40-CH_3), 26.5 (tail 4-CH_2), 26.6−26.7 (m, tail 8-, 12-, 16-, 20-, 24-, 28-, 32-, and 36-CH_2), 39.7 (m, tail 5-, 9-, 13-, 17-, 21-, 25-, 29-, 33- and 37-CH_2), 61.1 (5′-OCH_3), 61.1 (6′-OCH_3), 118.8 (tail 2-CH), 123.8 (tail 6-CH), 124.1−124.4 (m, tail 10-, 14-, 18-, 22-, 26-, 30-, 34-, and 38-CH), 131.2 (tail 39-C), 134.8−135.0 (tail 11-, 15-, 19-, 23-, 27-, 31-, 35-C), 135.2 (tail 7-C), 137.6 (tail 3-C), 138.8 (3-C), 141.6 (2-C), 144.2 (5-CO), 144.3 (6-CO), 183.9 (1-C=O), 184.7 (4-C=O).

[5-^{13}C]Ubiquinone-10 (1a): The procedure described above was performed with [3-^{13}C]ubiquinone-0 (**2a**; 0.21 g, 1.15 mmol) in dichloromethane (12 mL), silver oxide (0.64 g, 2.76 mmol), freshly distilled boron trifluoride−diethyl ether (0.85 mL, 6.91 mmol), tributyl(decaprenyl)tin (**28**; 1.65 g 1.70 mmol) in dichloromethane (10 mL), and KF (4 g) to obtain a crude yield of 1.71 g. After purification on silica, pure [5-^{13}C]ubiquinone-10 (**1a**; 0.37 g, 0.43 mmol, 37%) was obtained together with pure [3-^{13}C]ubiquinone-0 (**2a**; 0.12 g 0.66 mmol, 57%). ^{1}H NMR (600 MHz): δ = 3.98 (d, $^3J_{C-H}$ 3.5 Hz, 3 H, 5′-OCH_3). ^{13}C NMR (150 MHz): δ = 11.9 (d, $^3J_{C-C}$ 1.6 Hz, 3′-CH_3), 61.1 (s, 6′-OCH_3), 61.1 (d, $^2J_{C-C}$ 2.0 Hz, 5′-OCH_3), 138.8 (d, $^2J_{C-C}$ 16.2 Hz, 3-C), 141.6 (d, $^3J_{C-C}$ 3.0 Hz, 2-C), 144.2 (5-^{13}CO), no signal found for 6-CO, 183.9 (d, $^2J_{C-C}$ 9.9 Hz, 1-C=O), 184.7 (d, $^1J_{C-C}$ 59.1 Hz, 4-C=O).

[6-^{13}C]Ubiquinone-10 (1b): The procedure described above was performed with [2-^{13}C]ubiquinone-0 (**2b**; 0.26 g, 1.42 mmol) in dichloromethane (14 mL), silver oxide (0.73 g, 3.15 mmol), freshly distilled boron trifluoride−diethyl ether (0.97 mL, 7.89 mmol), tributyl(decaprenyl)tin (**28**; 1.88 g, 1.94 mmol) in dichloromethane (12 mL), and KF (4 g) to obtain a crude yield of 2.06 g. After purification on silica, pure [6-^{13}C]ubiquinone-10 (**1b**; 0.48 g, 0.56 mmol, 39%) was obtained together with pure [2-^{13}C]ubiquinone-0 (**2b**; 0.13 g, 0.71 mmol, 50%). ^{1}H NMR (600 MHz): δ = 3.99 (d, $^3J_{C-H}$ 3.5 Hz, 3 H, 6′-OCH_3). ^{13}C NMR (150 MHz): δ = 11.9 (s, 3′-CH_3), 25.3 (d, $^3J_{C-C}$ 1.6 Hz, tail 1-CH_2), 61.1 (s, 5′-OCH_3), 61.1 (d, $^2J_{C-C}$ 4.5 Hz, 6′-OCH_3), 138.8 (d, $^3J_{C-C}$ 3.0 Hz, 3-C), 141.6 (d, $^2J_{C-C}$ 15.9 Hz, 2-C), no signal found for 5-CO, 144.3 (6-^{13}CO), 183.9 (d, $^1J_{C-C}$ 58.9 Hz, 1-C=O), 184.7 (d, $^2J_{C-C}$ 9.9 Hz, 4-C=O).

Syntheses in the Preparation of [2-^{13}C]-Ubiquinone-0

Monomethyl 3-Methylglutarate: Methanol (4.71 mL, 116.3 mmol) was added to 3-methylglutaric anhydride (**11**; 12.44 g, 97.1 mmol). This was stirred for 45 min at 80 °C. After evaporation of the excess methanol under vacuum, 15.39 g (99%) of product was obtained. ^{1}H NMR: δ = 1.05 (d, $^3J_{H-H}$ 6.2 Hz, 3 H, CH_3), 2.4 (m, 5 H, 2CH_2 and CH), 3.68 (s, 3 H, OCH_3), 10.8 (br. s, OH). ^{13}C NMR: δ = 19.5 (CH_3), 26.9 (CH), 40.2 (2CH_2), 51.3 (OCH_3), 172.7 (C=O), 177.8 (C=O).

Methyl (4-Chloroformyl)-3-methylbutyrate (12): Thionyl chloride (17.6 mL, 203 mmol) was added to monomethyl 3-methylglutarate (15.39 g, 96.2 mmol). After stirring overnight, the excess thionyl chloride was evaporated under vacuum. Subsequent vacuum distillation (bp. 60 °C at 0.3 Torr) yielded pure product (16.3 g, 94%). ^{1}H NMR: δ = 1.08 (d, $^3J_{H-H}$ 6.5 Hz, 3 H, CH_3), 2.30 (dd, $^3J_{H-H}$ 7.2 Hz and $^2J_{H-H}$ 15.4 Hz, 1 H, CH_AH$_B$COOMe), 2.40 (dd, $^3J_{H-H}$ 6.2 Hz and $^2J_{H-H}$ 15.4 Hz, 1 H, CH$_A$$H_B$COOMe), 2.5 (m, 1 H, CH), 2.84 (dd, $^3J_{H-H}$ 7.2 Hz and $^2J_{H-H}$ 17.0 Hz, 1 H, CH_AH$_B$COCl), 3.07 (dd, $^3J_{H-H}$ 5.8 Hz and $^2J_{H-H}$ 17.0 Hz, 1 H, CH$_A$$H_B$COCl), 3.69 (s, 3 H, OCH_3). ^{13}C NMR: δ = 19.3 (CH_3),

27.7 (CH), 39.7 (2-CH_2), 51.6 (4-CH_2), 53.0 (OCH_3), 172.0 (C=O), 172.5 (C=O).

Methyl 3-Methyl-5-oxohexanoate: A solution of iodomethane (10.00 g, 4.39 mL, 70.5 mmol) in ether (20 mL) was added dropwise to magnesium turnings (1.58 g, 65.0 mmol) in refluxing ether (40 mL) in such a way as to maintain the ether at a gentle reflux. To make sure no magnesium was left, heating at reflux was continued for about 30 min (residual magnesium seriously reduces the yields of the subsequent reactions). The Grignard mixture was cooled down to 0 °C and dry $CdCl_2$ (8.00 g, 43.6 mmol) was added, followed by reflux for further 45 min. All the ether solvent was then evaporated under a flow of argon. The obtained gray, sandy substance was dispersed in toluene (100 mL) at room temperature, after which methyl (4-chloroformyl)-3-methylbutyrate (**12**; 9.01 mL) in toluene (10 mL) was added dropwise. After a further 2 h at reflux, the mixture was cooled down to room temperature, after which saturated NH_4Cl solution (30 mL) was added, followed by some 1 N HCl. After separation of the water layer, which was extracted twice with ether, the combined organic layers were washed with saturated $NaHCO_3$, NH_4Cl, and NaCl solutions and dried with $MgSO_4$. After evaporation under vacuum, the yellow liquid (9.65 g) was distilled under vacuum (0.3 Torr). The fraction collected at 60−70 °C gave methyl 3-methyl-5-oxohexanoate (7.34 g) as a colorless liquid, in a yield of 66% with regard to iodomethane. ^{1}H NMR: δ = 0.99 (d, $^3J_{H-H}$ 6.5 Hz, 3 H, 3′-CH_3), 2.14 (s, 3 H, 6-CH_3), 2.2−2.5 (m, 5 H, 2CH_2 and CH), 3.67 (s, 3 H, OCH_3). ^{13}C NMR: δ = 19.7 (3′-CH_3), 27.4 (3-CH), 30.2 (6-CH_3), 40.5 (CH_2), 49.8 (CH_2), 51.4 (OCH_3), 172.3 (1-C=O), 207.2 (5-C=O).

Methyl [6-^{13}C]-3-Methyl-5-oxohexanoate: The procedure described above was performed with [^{13}C]iodomethane (10.00 g, 70.0 mmol) in ether (20 mL), magnesium turnings (1.58 g, 65.0 mmol) in refluxing ether (50 mL), $CdCl_2$ (8.16 g, 44.5 mmol), and methyl 4-chloroformyl-3-methylbutyrate (**12**, 9.01 mL) in toluene (10 mL) to obtain 10.42 g of a yellow liquid, which, after vacuum distillation (60 to 70 °C, 0.3 Torr), yielded 7.64 g as a colorless liquid in a yield of 69% with regard to [^{13}C]iodomethane. ^{1}H NMR: δ = 2.14 (d, $^1J_{C-H}$ 127.4 Hz, 3 H, $^{13}CH_3$). ^{13}C NMR: δ = 30.3 ($^{13}CH_3$).

5-Methyl-1,3-cyclohexanedione (13): Potassium (2.06 g, 52.7 mmol) was added to *tert*-butyl alcohol (345 mL). After all the potassium had reacted, methyl 3-methyl-5-oxohexanoate (6.88 g, 43.5 mmol) was added and the mixture was stirred overnight. The *tert*-butyl alcohol was evaporated under vacuum and the obtained brown solid was redissolved in THF (200 mL) and concentrated HCl (4.4 mL), diluted to 40 mL with water. The mixture was saturated with NaCl, after which the water layer was separated and extracted with THF (100 mL). The combined organic phases were dried carefully with $MgSO_4$. After evaporation of the solvent, a crude yield of 6.20 g was obtained. The crude product was dissolved in warm acetone (20 mL). After overnight crystallization at −20 °C, a first fraction of pure product (2.41 g) was obtained. Additional product (0.43 g) could be obtained from the residue in a second round of crystallization, bringing the total yield to 2.84 g (53%). The product was obtained mostly in its enol form. ^{1}H NMR: δ = 1.09 (d, $^3J_{H-H}$ 5.8 Hz, 3 H, CH_3 enol form), 1.12 (d, $^3J_{H-H}$ 6.2 Hz, 3 H, CH_3 keto form), 2.0−2.8 (several m, 10 H, 2 CH_2 and 5-CH for both enol and keto forms), 3.38 (s, 2 H, 2-CH_2 keto form), 5.47 (s, 1 H, 2-CH enol form), 9.8 (br. s, OH enol form). ^{13}C NMR: keto: δ = 19.7 (CH_3), 25.9 (5-CH), 48.0 (2CH_2), 57.7 (2-CH), 203.8 (2 C=O); enol: δ = 20.7 (CH_3), 28.8 (5-CH), 40.5 (CH_2), 51.3 (CH_2), 103.8 (2-CH), 172.7 (3-COH), 191.6 (1-C=O).

[2-^{13}C]-5-Methyl-1,3-cyclohexanedione (13b): The procedure described above was performed with *tert*-butyl alcohol (400 mL), potassium (2.09 g, 53.5 mmol), and methyl [6-^{13}C]-3-methyl-5-oxohexanoate (7.64 g, 48.1 mmol) for the reaction, and THF (200 mL) and concentrated HCl (4.88 mL), diluted to 40 mL with water, for the workup procedure, to obtain a crude yield of 6.86 g. Recrystallization in 20 mL warm acetone yielded a first fraction (4.71 g) and a second fraction (0.18 g) of pure [2-^{13}C]-5-methylcyclohexane-1,3-dione (**13b**), bringing the total yield to 4.89 g (80%). The product was obtained mostly in its enol form. ^{1}H NMR: δ = 3.38 (d, $^1J_{C-H}$ 130.1 Hz, 2 H, $^{13}CH_2$ keto form), 5.48 (d, $^1J_{C-H}$ 160.3 Hz, 1 H, ^{13}CH enol form). ^{13}C NMR: δ = 57.7 ($^{13}CH_2$ keto form), 103.9 (^{13}CH enol form).

2-Chloro-5-methylcyclohexane-1,3-dione (14): 5-Methyl-1,3-cyclohexanedione (**13**; 5.0 g, 39.7 mmol) was dissolved in THF (15 mL) and water (50 mL), which was subsequently cooled to 0 °C. After dropwise addition of chloramine-T (11.2 g, 49.2 mmol) in water (75 mL), this mixture was stirred for 30 min and then filtered. The residue was washed with water (5 mL). The filtrate was acidified to pH = 2 with concentrated HCl and the acidic mixture was saturated with NaCl. This mixture was extracted four times with THF and the collected organic phases were dried thoroughly with $MgSO_4$. After concentration under vacuum, a yield of 4.52 g (71%) was obtained. The product was obtained in its enol form. ^{1}H NMR ([D_6]acetone): δ = 1.12 (d, $^3J_{H-H}$ 5.5 Hz, 3 H, CH_3), 2.3 (m, 3 H, 5-CH and 4-CH_2), 2.6 (m, 2 H, 6-CH_2).

[2-^{13}C]-2-Chloro-5-methyl-1,3-cyclohexanedione (14b): The procedure described above was performed with [2-^{13}C]-5-methyl-1,3-cyclohexanedione (**13b**; 4.71 g, 37.1 mmol) in THF (7 mL) and water (35 mL), and chloramine-T (10.55 g, 46.3 mmol) in water (69 mL) to obtain 4.02 g (67%) of product. ^{13}C NMR ([D_6]acetone): δ = 109.2 (^{13}CCl).

2-Chloro-3-methoxy-5-methyl-2-cyclohexenone (15): Trimethyl orthoformate (5 mL, 45.7 mmol) and concentrated HCl (6 drops) were added to 2-chloro-5-methyl-1,3-cyclohexanedione (**14**; 4.52 g, 28.2 mmol) in methanol (25 mL). After overnight stirring, the solvent was evaporated under vacuum, and a yield of 4.82 g (98%) was obtained. ^{1}H NMR: δ = 1.15 (d, $^3J_{H-H}$ 5.8 Hz, 3 H, CH_3), 2.3 (m, 3 H, 4-CH_2 and 5-CH), 2.7 (m, 2 H, 6-CH_2), 3.96 (s, 3 H, OCH_3). ^{13}C NMR: δ = 20.7 (CH_3), 27.8 (5-CH), 34.2 (CH_2), 44.7 (CH_2), 56.3 (OCH_3), 111.1 (CCl), 170.4 (3-CO), 190.7 (C=O).

[2-^{13}C]-2-Chloro-3-methoxy-5-methyl-2-cyclohexenone (15b): The procedure described above was performed with [2-^{13}C]-2-chloro-5-methyl-1,3-cyclohexanedione (**14b**; 4.02 g, 24.9 mmol) in methanol (25 mL), trimethyl orthoformate (3.8 mL, 34.7 mmol), and concentrated HCl (5 drops) to obtain a yield of 4.34 g (99%). ^{13}C NMR: δ = 111.1 (^{13}CCl).

2-Chloro-3-methoxy-5-methylphenol (16): A butyllithium solution in hexane (1.6 M, 5.1 mL, 8.16 mmol) was added by syringe at −15 °C to diisopropylamine (1.50 mL, 10.7 mmol), dissolved in THF (22 mL), followed after 10 min by dropwise addition of 2-chloro-3-methoxy-5-methyl-2-cyclohexenone (**15**; 0.87 g, 4.99 mmol) in THF (14 mL). After 5 min, chlorotrimethylsilane (4.56 mL, 35.9 mmol) was added and the mixture was allowed to warm to room temperature over 1 h. The reaction mixture was then heated to reflux, and iodine (1.52 g, 5.99 mmol) in THF (22 mL) was added dropwise under illumination with a 100-W tungsten lamp, followed by 3 h at reflux under illumination. After the mixture had cooled to room temperature, some saturated sodium dithionite solution was added and the water layer was separated and extracted three times with ether. The combined organic phases were dried with $MgSO_4$ and the solvent was evaporated under vacuum. The resulting mixture (1.20 g) was dissolved in undistilled methanol (15 mL) and KF (2 g)

was added, followed by 30 min of stirring. Some water was added to dissolve the salts and the water layer was separated and extracted three times with ether. The combined organic phases were washed with saturated NaCl solution and dried with $MgSO_4$. After concentration under vacuum, crude product (0.77 g, 90%) was obtained. ^{1}H NMR: δ = 2.29 (s, 3 H, CH_3), 3.86 (s, 3 H, OCH_3), 5.6 (br. s, OH), 6.38 (s, 1 H, CH), 6.54 (s, 1 H, CH).

[2-^{13}C]-2-Chloro-3-methoxy-5-methylphenol (16b): The procedure described above was performed with diisopropylamine (7.43 mL, 53.0 mmol) in THF (110 mL), a butyllithium solution in hexane (1.6 M, 25.3 mL, 40.5 mmol), [2-^{13}C]-2-chloro-3-methoxy-5-methyl-2-cyclohexenone (**15b**; 4.31 g, 24.6 mmol) in THF (90 mL), chlorotrimethylsilane (4.56 mL, 35.9 mmol), and iodine (7.53 g, 29.7 mmol) in THF (110 mL) for the reactions. The obtained product (7.33 g) was worked up with KF (6 g) in undistilled methanol (55 mL) to obtain crude product (4.07 g, 96%). ^{1}H NMR: δ = 6.38 (d, $^3J_{C-H}$ 8.6 Hz, 1 H, CH), 6.54 (d, $^3J_{C-H}$ 7.9 Hz, 1 H, CH). ^{13}C NMR: δ = 105.4 (^{13}CCl).

Fremy's Salt − Potassium Nitrosodisulfonate [$K_2(SO_3)_2NO$]: $NaNO_2$ (10.35 g, 150 mmol) was dissolved in water (30 mL) in a 1000-mL round-bottomed flask, which was cooled in an ice-bath during the whole procedure. To this solution was added crushed ice (60 g) and $NaHSO_3$ (10.5 g, 101 mmol). After addition of glacial acetic acid (6 mL, 104 mmol), stirring was continued for 4 min, during which the solution became yellow. Concentrated ammonia (7.5 mL, 125 mmol) was then added, followed by addition of some more crushed ice. Some crushed ice should from now on always be present in the flask. After dropwise addition of $KMnO_4$ solution (0.2 M, 120 mL, 24.0 mmol), stirring was continued for 20 min. The MnO_2 precipitate was removed by paper filtration. The filtrate was collected in a 1-L Erlenmeyer flask and kept on ice. From this point on, no further crushed ice was added. To precipitate the Fremy's salt, KCl was added until an orange precipitate formed. The precipitate was collected in a fritted glass funnel and subsequently washed with saturated KCl solution (30 mL) containing 5% concentrated ammonia, with methanol (30 mL) containing 5% concentrated ammonia, and finally with pure acetone (40 mL). A slightly moist orange salt (about 12 g) was obtained, and was used without further drying or purification. It was kept at 4 °C in a well-sealed jar with a drop of concentrated ammonia on the inside of the lid.

2-Chloro-3-methoxy-5-methyl-*para*-benzoquinone (17): A buffer was prepared from K_2HPO_4 (5.0 g, 28.7 mmol) in water (200 mL) and brought to pH = 6.5 with HCl. To this buffer was added Fremy's salt (7.5 g, prepared as described above) and 3-methoxy-5-methylphenol (**16**; 1.02 g, 5.91 mmol), dissolved in ether (10 mL). After this mixture had been stirred overnight, ether (100 mL) was added. The water layer was separated and extracted four times with ether. The combined organic phasees were washed with saturated NaCl solution and dried with $MgSO_4$. After evaporation of the solvent under vacuum, crude product (0.60 g) was obtained. After purification on silica with 15% ether/85% PE as eluent, a yield of 0.28 g [26% based on 2-chloro-3-methoxy-5-methyl-2-cyclohexenone (**15**)] was obtained. ^{1}H NMR: δ = 2.08 (d, $^4J_{H-H}$ 1.7 Hz, 3 H, CH_3), 4.19 (s, 3 H, OCH_3), 6.67 (q, $^4J_{H-H}$ 1.7 Hz, 1 H, CH).

[2-^{13}C]-2-Chloro-3-methoxy-5-methyl-*para*-benzoquinone (17b): The procedure described above was performed with K_2HPO_4 (20 g, 115 mmol), water (800 mL), Fremy's salt (30 g), and [2-^{13}C]-3-methoxy-5-methylphenol (**16b**; 4.07 g, 23.5 mmol) in ether (40 mL) to obtain crude product (2.91 g), which yielded pure product 1.61 g {1.61 g, 35% based on [2-^{13}C]-2-chloro-3-methoxy-5-methyl-2-cyclohexenone (**15b**)} after purification on silica. ^{1}H NMR: δ = 6.67 (dq, $^4J_{H-H}$ 1.7 Hz and $^3J_{C-H}$ 7.6 Hz, 1 H, CH). ^{13}C NMR: δ = 125.0 (^{13}CCl).

Diels−Alder Adduct of Cyclopentadiene and [2-^{13}C]-2-Chloro-3-methoxy-5-methyl-*para*-benzoquinone

[2-^{13}C]-2-Chloro-3-methoxy-4aα-methyl-4a,5,8,8a-tetrahydro-5α,8α-methano-1,4-naphthoquinone: [2-^{13}C]-2-Chloro-3-methoxy-5-methyl-*para*-benzoquinone (**17b**; 0.99 g 5.31 mmol) and freshly distilled cyclopentadiene (5 mL) were dissolved in dichloromethane (10 mL). After this mixture had been stirred for 40 h, the solvent was evaporated under vacuum and replaced by PE (10 mL). The excess cyclopentadiene was removed in a flash column, with PE as eluent. The product was removed from the flash column by elution with ether. After evaporation of the solvent under vacuum, pure product (1.34 g, 100%) was obtained. ^{1}H NMR: δ = 1.52 (s, 3 H, 4aα-CH_3), 1.59 (d, $^2J_{H-H}$ 9.3 Hz, 1 H, CH_AH$_B$), 1.71 (d, $^2J_{H-H}$ 9.3 Hz, 1 H, CH$_A$$H_B$), CH_2), 2.97 (dd, $^3J_{H-H}$ 3.8 Hz and $^3J_{C-H}$ 2.1 Hz, 1 H, 8a-CH), 3.11 (br. s, 1 H, CH), 3.48 (br. s, 1 H, CH), 4.11 (s, 3 H, OCH_3), 6.04 (dd, $^3J_{H-H}$ 3.1 Hz and 5.8 Hz, 1 H, CH=*CH*), 6.16 (dd, $^3J_{H-H}$ 3.1 Hz and 5.8 Hz, 1 H, *CH*=CH). ^{13}C NMR: δ = 26.2 (4aα-CH_3), 46.2 (CH_2), 48.8 (CH), 53.1 (CH), 57.1 (OCH_3), 57.5 (4a-C), 61.3 (8a-CH), 131.0 (^{13}CCl), 135.0 (CH=*CH*), 138.0 (*CH*=CH).

3-Methoxy-6-methyl-2-cyclohexenone (7)

4-Methyl-5-oxohexanoic Acid: Sodium (0.42 g, 18.3 mmol) was added to methanol (100 mL). After all the sodium had reacted, ethyl 2-methylacetoacetate (**18**; 10.00 mL, 70.7 mmol) was added, followed by dropwise addition of methyl acrylate (**19**; 7.00 mL, 77.7 mmol) in methanol (15 mL). After overnight stirring, KOH (10 g, 178 mmol) in water (15 mL) was added. After further overnight stirring, concentrated aqueous HCl (15 mL) in water (5 mL) was added carefully, followed by 1 h of stirring at 65 °C. Additional concentrated aqueous HCl (2 mL) in water (5 mL) was then added and stirring was continued for another 1.5 h at 65 °C, followed by overnight stirring at room temperature. After evaporation of the solvent, the residue was dissolved in ether and dried with $MgSO_4$. A crude yield of 7.63 g (75%) was obtained. ^{1}H NMR: δ = 1.14 (d, $^3J_{H-H}$ 6.9 Hz, 3 H, 4′-CH_3), 1.68 (m, 1 H, 3-CH_AH$_B$), 1.99 (m, 1 H, 3-CH$_A$$H_B$), 2.18 (s, 3 H, 6-$CH_3$), 2.36 (t, $^3J_{H-H}$ 7.2 Hz, 2 H, 2-CH_2), 2.62 (m, 1 H, 4-CH). ^{13}C NMR: δ = 16.1 (4′-CH_3), 27.0 (3-CH_2), 28.1 (6-CH_3) 31.3 (2-CH_2), 45.8 (4-CH), 178.5 (COOH), 212.2 (5-C=O).

Methyl 4-Methyl-5-oxohexanoate (21): Molecular sieves (3 Å, 5 g) and *p*-toluenesulfonic acid (0.1 g, 0.53 mmol) were added to 4-methyl-5-oxohexanoic acid (7.41 g) in methanol (50 mL). After two nights of stirring, the mixture was filtered through Hyflo Super Cel and the solvent was evaporated under vacuum. After vacuum distillation (55−70 °C, 0.3 Torr), 6.36 g (78%) of product was obtained. ^{1}H NMR: δ = 1.12 (d, $^3J_{H-H}$ 7.2 Hz, 3 H, 4′-CH_3), 1.72 (m, 1 H, 3-CH_AH$_B$), 1.99 (m, 1 H, 3-CH$_A$$H_B$), 2.16 (s, 3 H, 6-$CH_3$), 2.31 (t, $^3J_{H-H}$ 7.2 Hz, 2 H, 2-CH_2), 2.60 (m, 1 H, 4-CH), 3.67 (s, 3 H, OCH_3). ^{13}C NMR: δ = 16.1 (4′-CH_3), 27.3 (3-CH_2), 28.0 (6-CH_3), 31.4 (2-CH_2), 45.9 (4-CH), 51.4 (OCH_3), 173.4 (1-C=O), 211.6 (5-C=O).

6-Methyl-1,3-cyclohexanedione (22): Potassium (0.56 g, 14.3 mmol) was added to *tert*-butyl alcohol (100 mL). After all the potassium had reacted, methyl 4-methyl-5-oxohexanoate (**21**; 2.04 g, 12.9 mmol) was added and the mixture was stirred overnight. The *tert*-butyl alcohol was evaporated under vacuum and the obtained brown solid was dissolved in THF (54 mL) and water (11 mL), acidified with concentrated HCl (1.32 mL). The mixture was satur-

ated with NaCl. The water layer was separated and extracted with THF (100 mL). The combined organic phasees were dried carefully with $MgSO_4$. After evaporation of the solvent, a crude yield of 1.77 g (109%) was obtained. The product was obtained as a mixture of a keto form and two enol forms. ^{1}H NMR: δ = 1.20 (m, 9 H, 3 CH_3 keto and enol forms), 1.7−2.7 (several m, 15 H, 2-CH_2 and 5-CH for both enol and keto forms), 3.37 (s, 2 H, 2-CH_2 keto form), 5.74 (s, 2 H, 2-CH enol forms), 10.1 (br. s, OH enol forms).

3-Methoxy-6-methyl-2-cyclohexenone (7): Trimethyl orthoformate (3 mL, 27.4 mmol) and concentrated HCl (5 drops) were added to 6-methyl-1,3-cyclohexanedione (**22**; 1.77 g, 14.0 mmol) in methanol (25 mL). After overnight stirring, the solvent was evaporated under vacuum and a crude yield of 1.95 g (99%) was obtained. The two isomers (**7** + **25**) were separated on silica, and 3-methoxy-6-methyl-2-cyclohexenone (**7**; 0.41 g, 21%) and 3-methoxy-4-methyl-2-cyclohexenone (**25**; 0.36 g, 18%) were obtained. ^{1}H NMR: δ = 1.15 (d, $^3J_{H-H}$ 6.9 Hz, 3 H, CH_3), 1.7−2.5 (several m, 5 H, 2CH_2 and 6-CH), 3.69 (s, 3 H, OCH_3), 5.34 (s, 1 H, 2-CH). ^{13}C NMR: δ = 15.0 (CH_3), 27.8 (5-CH_2), 28.9 (4-CH_2), 39.8 (6-CH), 55.3 (OCH_3), 101.2 (2-CH), 177.3 (CO), 201.3 (C=O).

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[1] G. Feher, J. P. Allen, M. Y. Okamura, D. C. Rees, *Nature* **1989**, *339*, 111−116.
[2] W. B. S. van Liemt, W. F. Steggerda, R. Esmeijer, J. Lugtenburg, *Recl. Trav. Chim. Pays-Bas* **1994**, *113*, 153−161.
[3] W. B. S. van Liemt, G. J. Boender, P. Gast, A. J. Hoff, J. Lugtenburg, *Biochemistry* **1995**, *34*, 10229−10236.
[4] R. Brudler, H. J. M. de Groot, W. B. S. van Liemt, W. F. Steggerda, R. Esmeijer, P. Gast, A. J. Hoff, J. Lugtenburg, K. Gerwert, *EMBO J.* **1994**, *13*, 5523−5530.
[5] J. Breton, C. Boullais, J. R. Burie, E. Nabedryk, C. Mioskowski, *Biochemistry* **1994**, *33*, 14378−14386.
[6] J. R. Burie, C. Boullais, M. Nonella, M. Mioskowski, E. Nabedryk, J. Breton, *J. Chem. Phys. B* **1997**, *101*, 6607−6617.
[7] J. S. van den Brink, A. P. Spoyalov, P. Gast, W. B. S. van Liemt, J. Raap, J. Lugtenburg, A. J. Hoff, *FEBS Lett.* **1994**, *353*, 273−276.
[8] M. Rohrer, P. Gast, K. Möbius, T. F. Prisner, *Chem. Phys. Lett.* **1996**, *259*, 523−530.
[9] S. Ermakova-Gerdes, V. Vermaas, *Biochemistry* **1998**, *37*, 11569−11578.
[10] J. Lugtenburg, H. J. M. de Groot, *Photosynth. Res.* **1998**, *55*, 241−245.
[11] H. J. M. de Groot, R. Gebhard, K. van der Hoef, A. J. Hoff, J. Lugtenburg, C. A. Violette, H. A. Frank, *Biochemistry* **1992**, *31*, 12446−12450.
[12] M. R. Fisher, H. J. M. de Groot, J. Raap, C. Winkel, A. J. Hoff, J. Lugtenburg, *Biochemistry* **1992**, *31*, 11038−11049.
[13] C. Boullais, J. Breton, E. Nabedryk, C. Mioskowski, *Tetrahedron* **1997**, *53*, 2505−2512.
[14] L. Syper, K. Kloc, J. Mlochowski, *Tetrahedron* **1980**, *36*, 123−129.
[15] A. Rüttiman, P. Lorenz, *Helv. Chim. Acta* **1990**, *73*, 790−796.
[16] E. Keinan, D. Eren, *J. Org. Chem.* **1987**, *52*, 3872−3875.
[17] Y. Fujita, M. Ishiguro, T. Onishi, T. Nishida, *Synthesis* **1981**, 469−471.
[18] H. Dieks, K. Beyer, *J. Labelled Comp. Radiopharm.* **1990**, *28−29*, 1093−1107.
[19] A. Duralski, A. Watts, *Tetrahedron Lett.* **1992**, *33*, 4983−4984.
[20] E. M. M. van den Berg, F. J. H. M. Jansen, A. T. W. J. de Goede, A. U. Baldew, J. Lugtenburg, *Recl. Trav. Chim. Pays-Bas* **1990**, *109*, 287−297.
[21] H. Zimmer, D. Lankin, S. Horgan, *Chem. Rev.* **1971**, *71*, 229−246.
[22] A. Fischer, G. Henderson, *Synthesis* **1985**, 641−643.
[23] L. Syper, K. Kloc, J. Mlochowski, *Tetrahedron* **1980**, *36*, 123−129.
[24] Y. Fujita, M. Ishiguro, T. Onishi, T. Nishida, *Bull. Chem. Soc. Jpn.* **1982**, 1325−1326.
[25] B. Liu, L. Gu, J. Zhang, *Recl. Trav. Chim. Pays-Bas* **1991**, *110*, 104−110.
[26] A. Merz, M. Rausel, *Synthesis* **1993**, 797−802.
[27] K. Sato, S. Inoue, R. Yamaguchi, *J. Org. Chem.* **1972**, *37*, 1889−1892.
[28] K. Sato, S. Inoue, A. Onishi, N. Uchida, N. Minowa, *J. Chem. Soc., Perkin Trans. 1* **1981**, 761−769.
[29] Y. Naruta, K. Maruyama, *Chem. Lett.* **1979**, 885−888.
[30] Y. Naruta, K. Maruyama, *Org. Synth.* **1993**, *71*, 125−132.
[31] J. Cason, *Org. Synth. Coll. Vol. III*, **1963**, 169−171.
[32] R. Shepherd, A. White, *J. Chem. Soc., Perkin Trans. 1* **1987**, 2153−2155.
[33] A. S. Kotnis, *Tetrahedron Lett.* **1991**, *32*, 3441−3444.
[34] S. M. Hannick, Y. Kishi, *J. Org. Chem.* **1983**, *48*, 3833−3835.
[35] C. H. Eichinger, J. H. Bowie, *Org. Mass Spectrom.* **1987**, *22*, 103−108.
[36] J. J. Cappon, J. Baart, G. A. M. van der Walle, J. Raap, J. Lugtenburg, *Recl. Trav. Chim. Pays-Bas* **1991**, *110*, 158−166.
[37] Calculated with the program *LABEL* by: S. Schripsema, D. Dagnino, The Technical University of Denmark, **1993**, version 4.1.
[38] W. B. S. van Liemt, *The functionally Asymmetric Q_A Ubiquinone-10 Binding in the Bacterial Photosynthetic Reaction Centre*, Thesis, Leiden University, **1994**.
[39] J. H. Bowie, D. W. Cameron, R. G. F. Giles, D. H. Williams, *J. Chem. Soc.* **1966**, 335−339.
[40] H. O. Kalinowski, S. Berger, S. Braun, *^{13}C NMR Spektroskopie*, Georg Thieme Verlag, Stuttgart, New York, **1984**.
[41] M. Y. Okamura, R. A. Isaacson, G. Feher, *Proc. Natl. Acad. Sci. U. S. A.* **1975**, *72*, 3491−3495.
[42] C. Winkel, E. G. Buitenhuis, J. Lugtenburg, *Recl. Trav. Chim. Pays-Bas* **1989**, *108*, 51−56.
[43] R. Muraca, J. Whittick, G. Doyle Daves, P. Friis, K. Folkers, *J. Am. Chem. Soc.* **1967**, *89*, 1505−1508.
[44] G. Waller, *Biochemical Applications of Mass Spectrometry*, Wiley Interscience, **1972**, pp. 521−524.
[45] J. Breton, J.-P. Burie, C. Boullais, G. Berger, E. Nabedryk, *Biochemistry* **1994**, *33*, 12405−12415.
[46] H. H. Robinson, S. D. Kahn, *J. Am. Chem. Soc.* **1990**, *112*, 4728−4731.
[47] M. Nonella, C. Brändli, *J. Phys. Chem.* **1996**, *100*, 14549−14559.
[48] J. Breton, *Proc. Natl. Acad. Sci. U. S. A.* **1997**, *94*, 11318−11323.
[49] T. O. Yeates, H. Komiya, A. Chirino, D. C. Rees, J. P. Allen, G. Feher, *Proc. Natl. Acad. Sci. U. S. A.* **1988**, *85*, 7993−7997.
[50] C.-H. Chang, O. E. Kabbani, D. Tiede, J. Norris, M. Schiffer, *Biochemistry* **1991**, *30*, 5352−5360.
[51] B. Arnoux, J. F. Gaucher, A. Ducruix, F. Reiss-Husson, *Acta Crystallogr., Sect. D: Biol. Chrystallogr.* **1995**, *51*, 368−379.
[52] J. Breton, J.-P. Burie, C. Berthomieu, G. Berger, E. Nabedryk, *Biochemistry* **1994**, *33*, 4953−4965.
[53] B.-J. Rossum, *Structure refinement of photosynthetic components with multidimensional MAS NMR dipolar correlation spectroscopy*, Thesis, Leiden University, **2000**, p. 118−134.

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