Synthesis and Spectroscopic Characterization of 1-^{13}C- and 4-^{13}C-Plastoquinone-9

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This paper presents the synthesis of 1-^{13}C- and 4-^{13}C-plastoquinone-9 and their characterization with NMR spectroscopy and mass spectrometry. The synthetic scheme has been further adapted to introduce ^{13}C-labeled plastoquinones on all individual and on each combination of positions in the quinone ring. Also a two-step scheme is disclosed to prepare unlabeled plastoquinone-9.

Introduction

Plastoquinone-9 (2,3-dimethyl-5-solanesyl-benzoquinone (Figure 1; Solanesyl = 3,7,11,15,19,23,27,31,35,-nonamethyl-2,6,10,14,18,22,26,30,34,-hexatriacontanonaenyl) plays an important role in the initial redox chemistry and electron transport of the oxygenic photosynthesis of plants, algae and cyanobacteria. In these organisms photosynthesis is performed by two photosystems (PS 1 and PS 2) connected in series through which (sun) light is transformed into energy-rich molecules. These molecules are subsequently used by almost all organisms for their energy and structural requirements and thus oxygenic photosynthesis provides the basis for life on Earth.

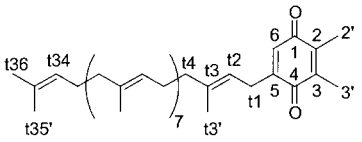

Figure 1. Structure and numbering of plastoquinone-9.

In plants, algae and cyanobacteria photosynthesis starts in photosystem 2, where light energy is used to oxidize water to oxygen and reduce plastoquinone to plastoquinol. The electrons stored on the plastoquinones are passed on through an electron transport chain to photosystem 1 and are subsequently used in the build up of the energy-rich molecules.

Photosystem 2 consists of a reaction center core, an oxygen evolving complex and a light harvesting complex. Recently its crystal structure was published which confirmed the already suspected near twofold symmetry of the eight cofactors building up the reaction center core.[1,2] These cofactors are two plastoquinones, an Fe^{2+}-ion, two pheophytins, two accessory chlorophylls and the special pair (P860) consisting of two chlorophylls. Despite this symmetry the two plastoquinones have a different function. The plastoquinone in the Q_A-site acts as a one-electron gate only and is tightly bound to the protein. It receives an electron from the photo-excited special pair (P860*), forming a Q_A-radical anion/$P860^+$ charge separated state. The electron is subsequently transferred via the Fe^{2+}-atom to the plastoquinone at the Q_B-site. In the meantime the oxidized special pair ($P860^+$) receives a new electron from the oxygen evolving complex, thereby gradually oxidizing water to oxygen. The Q_B-radical anion accumulates a second electron in the next photochemically driven step and takes up two protons from the inside of the thylakoid membrane. It then leaves the reaction center as plastoquinol and is replaced by a new plastoquinone from the quinone pool in the membrane.

The reaction center core of photosystem 2 resembles the well established structure of the photosynthetic reaction center of *Rhodobacter sphaeroides*,[3] because of the similar arrangement of the related cofactors and the similar amino acid sequences. The special role of the two plastoquinone-9 molecules also closely resembles the role of the two ubiquinone-10 molecules.[4] The latter has been under thorough investigation in the last decade.[5–9,11,12]

The different behavior of the plastoquinones in the Q_A- and Q_B-sites of photosystem 2 can only be induced by different protein-plastoquinone interactions. These interactions might be similar as previously found for ubiquinones

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in bacterial reaction centers of *Rhodobacter sphaeroides*. Here a strongly asymmetric dynamic binding of the protein with the Q_A-ubiquinone-10 was discovered,[5−9,11] by using MAS ^{13}C NMR, ESR and FTIR spectroscopy together with 1-, 2-, 3-, 3′-, 4-, 5- and 6-^{13}C-ubiquinone-10 and $(^{13}CH_3O)_2$-ubiquinone-10 obtained by total-synthesis.[10,11]

To investigate the behavior of the Q_A plastoquinone-9 at atomic detail a three-step strategy is implemented. First, specifically isotopically labeled plastoquinones are prepared by total synthesis. Second, these isotopically labeled plastoquinones will be incorporated into photosystem 2. Third, these specifically enriched proteins will be investigated with nonperturbing isotopically sensitive techniques, like SS NMR, ESR and FTIR spectroscopy.[12−14]

The convergent synthetic scheme for the synthesis of ^{13}C-labeled plastoquinones on any single and on every combination of positions is presented here as the first step of this strategy. Since the two carbonyl positions of ubiquinone-10 proved to have the most important interactions with the reaction center protein of *Rhodobacter sphaeroides* R-26, 1-^{13}C- and 4-^{13}C-plastoquinone-9 were synthesized first. Their synthesis, characterization with NMR spectroscopy and mass spectrometry are reported in detail.

Synthesis

The existing synthetic schemes for the synthesis of unlabeled plastoquinones begin with aromatic starting materials.[15−17] Unfortunately these cannot be easily modified for the ^{13}C-labeling of the plastoquinone ring on every single- and all combinations of positions. Since there are no suitable ^{13}C-labeled aromatic compounds commercially available, the synthetic scheme has to start with ^{13}C-enriched compounds such as potassium cyanide, iodomethane or acetic acid.

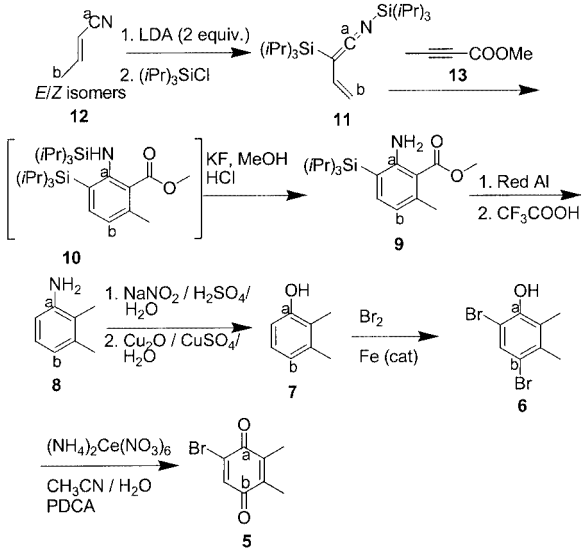
During the synthesis of ^{13}C-labeled compounds it is important to avoid synthetic steps in which dilution of the isotopic label can take place. In addition, scrambling of the label should be avoided by use of only fully regioselective reactions. To achieve this it is necessary that all reaction intermediates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

The first phase in the synthesis of ^{13}C-plastoquinone-9 is the synthesis of an aromatic compound from suitable and correctly ^{13}C-labeled compounds. We chose an Diels−Alder reaction previously used for the synthesis of antranilic acid.[18,19] The aromatic compound formed in this way has all the functionalities required to synthesize plastoquinone-0.[20−22] Since plastoquinone-0 is a symmetrical compound we needed to preserve a functionality that would make the attachment reaction of the solanesyl chain regioselective. We chose to induce asymmetry with a bromine, which made 6-bromo-2,3-dimethylbenzoquinone the central molecule in our synthetic scheme.

The second phase is the attachment of a poly-isoprenoid tail.[15,23−25] We used a synthesis with boron trifluoride−diethyl ether and solanesyltributyltin.[11,26] With this method no ^{13}C-labeled material is lost and any unchanged material can be easily recovered.[27,28] Finally the bromine that kept the plastoquinone-0 asymmetric has to be removed. All synthetic steps were of course first optimized by using unlabeled materials.

Synthesis of 6-Bromo-2,3-dimethylbenzoquinone

The first step in the synthesis of 6-bromo-2,3-dimethylbenzoquinone is the formation of the aromatic ring by a Diels−Alder reaction between butadiene ketenimine **11** and methyl 2-butynoate **13** in a sealed Carius tube (Scheme 1).[18] The air- and water-sensitive diene is synthesized from crotononitrile **12** by a reaction with LDA and triisopropylsilyl chloride. After a workup treatment with potassium fluoride and hydrochloric acid, aniline **9** is obtained, which already has the complete carbon skeleton of plastoquinone-0.



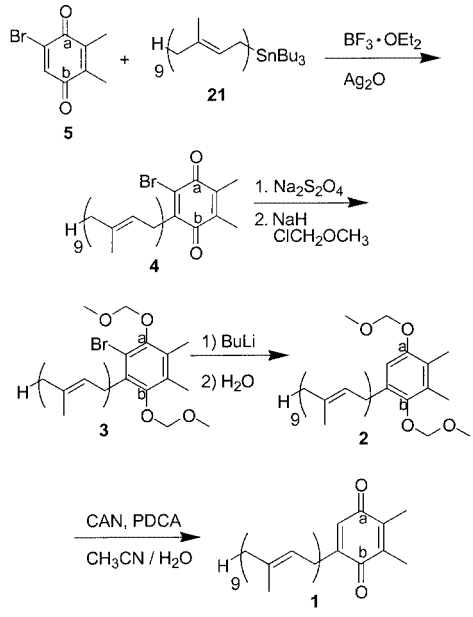
Scheme 1. Synthesis of 6-bromo-2,3-dimethylquinone (a and b indicate the positions of the ^{13}C-labels). PDCA = 2,6-pyridinedicarboxylic acid.

The ester group of aniline **9** is subsequently reduced with Red-Al in refluxing toluene. This reduction is possible because of the electron donating amine group. The remaining triisopropylsilyl group is removed with trifluoroacetic acid to give aniline **8**. After diazotation of this aniline and subsequent Cu^I-catalyzed hydrolysis,[29] the obtained phenol **7** is dibromated with bromine and a catalytic amount of iron. To oxidize dibromophenol **6** to quinone **5** a novel reaction with cerium ammonium nitrate (CAN) was applied, as we found that *p*-brominated phenols can be oxidized specifically to *p*-quinones in yields of around 60% with the mild oxidant CAN. Quinone **5** is actually an asymmetric brominated plastoquinone-0.

The above described synthesis was used to synthesize 0.12 g of 1-^{13}C-6-bromo-plastoquinone-0 **5a** and 0.17 g of 4-^{13}C-6-bromo-plastoquinone-0 **5b** in an overall yield of 3% in regard to the crotononitriles **12a** and **12b**, respectively.

Synthesis of Plastoquinone-9

The addition of the solanesyl tail is carried out by a boron trifluoride−diethyl ether catalyzed 1-4 addition between bromoquinone **5** and solanesyltributyltin **21** (Scheme 2).[16,23,25,28] Silver oxide is added to the reaction mixture to oxidize the initially formed 6-bromoplastoquinol-9 to quinone **4**. Any unchanged starting material can easily be recovered. The solanesyltributyltin **21** was synthesized from solanesol.[11]



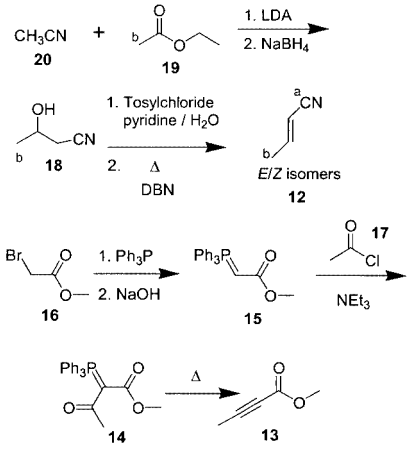
Scheme 2. Synthesis of plastoquinone-9 (a and b indicate the positions of the ^{13}C-labels).

The removal of the bromine from quinone **4** was performed in a few steps. After reduction of quinone **4** with sodium dithionite, the two hydroxyl groups where protected with MOM groups by a reaction with chloromethyl methyl ether and sodium hydride. To remove the bromine this protected bromine **3** was treated at low temperature with a butyllithium solution followed by quenching with water. Finally the di-MOM ether **2** was oxidized with CAN to plastoquinone-9 (**1**) in an overall yield of 32% (based on bromoquinone **5**).

The above described synthesis was used to synthesize 0.1 g of 1-^{13}C-plastoquinone-9 (**1a**) and 0.1 g of 4-^{13}C-plastoquinone-9 (**1b**) in an overall yield of 1% (based on K^{13}CN and ethyl 2-^{13}C-acetate **19b**, respectively).

Synthesis of the Diels−Alder Reagents

The first step in the synthesis of crotononitrile is a one pot reaction between the anion of acetonitrile **20** with ethyl acetate **19**, followed by reduction to alcohol **18** (Scheme 3).[30] This secondary alcohol is subsequently esterified with *p*-toluenesulfonyl chloride to obtain a better leaving group. An 1,2 elimination reaction is then performed on this tosyl ester by treatment with 1,5-diazabicyclo[4.3.0]non-5-ene at elevated temperature (±150 °C). In this way a mixture of *cis* and *trans* crotononitrile (**12**) is obtained in 54% overall yield.



Scheme 3. Synthesis of crotononitrile and methyl butynoate (a and b indicate the positions of the ^{13}C-labels)

The above described synthesis was used to synthesize 1.54 g of 4-^{13}C-crotononitrile (**12b**) in a yield of 30% [based on ethyl 2-^{13}C-acetate (**19b**)]. For the 1-^{13}C-crotononitrile **12a** a single substitution reaction between allyl chloride and potassium ^{13}C-cyanide with benzyltriethylammonium chloride as a phase catalyst in water/acetonitrile was used. This reaction produced 2.77 g of 1-^{13}C-crotononitrile (**12a**) in a yield of 54%.

The synthesis of methyl 2-butynoate (**13**) starts with a reaction of ethyl bromoacetate (**16**) and triphenylphosphane, followed by deprotonation with a sodium hydroxide solution (Scheme 3). The obtained phosphane **15** can subsequently react with acetyl chloride **17** to an initially formed phosphonium salt, which is immediately deprotonated by triethylamine to phosphane **14**. Under vacuum conditions and elevated temperature (±180 °C) phosphane **14** reacts in an internal Wittig reaction[31,32] to methyl 2-butynoate (**13**), which is collected in a cold trap in an overall yield of 60%.

Since acetonitrile (**20**), ethyl acetate (**19**), ethyl bromoacetate (**16**) and acetyl chloride (**17**) are available in all ^{13}C-enriched forms, all single and combinations of ^{13}C-labeled positions of the two Diels−Alder reagents (**12** and **13**) are accessible, which consequently makes it possible to synthesize ^{13}C-labeled plastoquinones **1** on all single and combinations of positions.

Spectroscopic Characterization

Mass Spectrometry

To confirm the identity and the degree of ^{13}C-enrichment of 1-^{13}C- and 4-^{13}C-6-bromo-plastoquinone-0 and 1-^{13}C- and 4-^{13}C-plastoquinone-9 mass spectrometry was performed.

Table 1. Elemental composition of the plastoquinones, their experimental and calculated exact masses and their ^{13}C-incorporation.

Compound	Empirical[a] formula	Exact mass (u)[a] found	calculated	^{13}C-label incorporation
6-Br-plastoquinone-0 (**5**)	$^{12}C_8H_7BrO_2$	213.9647	213.9630	–
1-^{13}C-6-Br-PQ-0 (**5a**)	$^{13}C_1{}^{12}C_7H_7BrO_2$	214.9652	214.9663	95.5 ± 3%
4-^{13}C-6-Br-PQ-0 (**5b**)	$^{13}C_1{}^{12}C_7H_7BrO_2$	214.9660	214.9663	99.1 ± 3%
plastoquinone-9 (**1**)	$^{12}C_{53}H_{80}O_2$	748.6189	748.6158	–
1-^{13}C-PQ-9 (**1a**)	$^{13}C_1{}^{12}C_{52}H_{80}O_2$	749.6255	749.6201	101.2 ± 3%
4-^{13}C-PQ-9 (**1b**)	$^{13}C_1{}^{12}C_{52}H_{80}O_2$	749.6248	749.6201	100.8 ± 3%

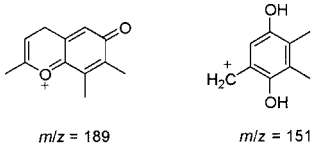
[a] For the calculations of the masses of the bromine containing compound ^{79}Br isotopes were taken into account.

The exact masses of these four compounds, obtained by double focus mass spectrometry, were found to be the same (within experimental error) as the calculated values. This confirmed their molecular formulas (Table 1).

To calculate the ^{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.[33] All incorporations found in this way, were close to the expected value of 99% (Table 1). These high incorporation figures confirm that no dilution of the label has taken place during the synthesis of labeled plastoquinone-9. The value for the 1-^{13}C-6-bromoplastoquinone-0 **1a** was found to be a bit lower, but since the 1-^{13}C-plastoquinone-9 **1a** is synthesized from 1-^{13}C-6-Bromoplastoquinone-0 **5a** we can safely assume that the incorporation of **5a** must be just as high as that of **1a**. The lower value is probably due to additional reduction of the quinone (Q) to the quinol (QH_2) in the mass spectrometer, which is a known problem in the mass spectrometry of quinones.[34−36] We found that the $[M + 2]^+$ signal increases in time. Using spectra taken immediately after the sample insertion allowed us to get spectra without an $[M + 2]^+$ signal. The ^{13}C-content was therefore calculated by comparison of labeled and unlabeled spectra taken immediately after insertion of the sample.

In the fragmentation pattern of 1-^{13}C- and 4-^{13}C-plastoquinones-9 the M^+ signal can be found at m/z = 749 whereas in unlabeled plastoquinone-9 it is found at 748. This is in accordance with literature.[34] At m/z [M − 15] a small signal is visible, probably due to loss of a methyl group. The next signals arise from breakdown of the isoprenoid chain in repetitive in steps of 68 au (C_5H_8). At m/z = 681 a small signal is found for the complete isoprenoid tail ($C_{45}H_{73}{}^+$), followed by the fragmentation of this chain by repetitive steps of 68 u.

At m/z = 257 a fragment is observed, which can be attributed to a $C_{17}H_{21}O_2{}^+$ fragment, which is the quinone ring with the first 9 carbon atoms of the isoprenoid-chain. For the two labeled compounds this fragment is correctly found at m/z = 258. In all three spectra a signal is observed at m/z = 203. This unlabeled signal can be attributed to a tail-fragment $C_{15}H_{23}{}^+$. Two relatively stable fragments are observed at m/z = 189 and 151. The first is a bicyclic oxonium ion $C_{12}H_{13}O_2{}^+$ and the second of a fragment $C_9H_{11}O_2{}^+$ (Figure 2),[35] both are found one mass unit higher for the labeled plastoquinones. At m/z = 135 a fragment is found, which does not include a label, probably due to $C_{10}H_{15}{}^+$ fragments from the isoprenoid tail. The fragmentation of plastoquinone-9 is conform that reported in literature.[34]



m/z = 189 m/z = 151

Figure 2. Structure of two stable fragments occurring in the mass spectrum of plastoquinone-9.

^{1}H NMR Spectroscopy

To confirm the structure, the position and the amount of ^{13}C-incorporation of the synthesized plastoquinones 600 MHz ^{1}H NMR spectra of 1-^{13}C- and 4-^{13}C-6-bromoplastoquinone-0 and 1-^{13}C- and 4-^{13}C-plastoquinone-9 were recorded. The spectra of the plastoquinones-9 are identical, apart from the expected additional couplings due to the coupling with the ^{13}C-isotope, to the spectra of low weight plastoquinones reported in literature.[16] This confirms the structure of our synthesized products. For plastoquinone-9 only 60 MHz NMR spectra were found in literature,[37] therefore a thorough description of the 600 MHz NMR spectra is reported here.

The 2′- and 3′-methyl groups of 6-bromoplastoquinone-0 are assigned to the signals at δ = 2.11 and 2.05 ppm, respectively, each as a quadruplet with a small five bond coupling of 1.2 Hz with each other. At δ = 7.25 ppm the signal of 5-H can be found as a singlet.

The proton spectra of the 1-^{13}C- and 4-^{13}C-6-bromoplastoquinones-0 show an extra coupling for the two methyl signals. Because it is known from literature that 3 bond coupling constants are bigger than 4 bond coupling constants,[38] the largest coupling constants found must be three bond coupling constants. Therefore the methyl group with the largest coupling constant between its protons and the ^{13}C-label must be the one closest to the label. The two methyl groups can then be assigned unequivocally. The signals at δ = 2.11 and 2.05 ppm can be assigned to the methyl groups attached to C-2 and to C-3, respectively, with their $^3J_{C-H}$ of 4.0 and 3.9 Hz. The signal of the proton on car-

bon-5 at δ = 7.25 ppm is also split for both labeled 6-bromoplastoquinones-0 due to the $^{13}C-H$ coupling with the label. The 1-^{13}C-label has a three bond coupling constant of 8.3 Hz and the 4-^{13}C-label has a two bond coupling constant of 0.7 Hz, which confirms the location of the labels.

The ring-proton signal can be used best to check the amount of ^{13}C-incorporation. In the labeled spectra no residual signals of unlabeled compounds could be observed. This means that the incorporation is indeed close to the expected value of 99%, as already observed by mass spectrometry.

The proton spectrum of plastoquinone-9 shows signals for the quinone ring (for numbering see Figure 1) at δ = 2.01 and 2.03 ppm for the 2′- and 3′-methyl group, respectively. The ring proton can be found at δ = 6.47 ppm as a broad triplet due to a coupling with the tail-1-methylene of 1.7 Hz. The remaining signals are assigned to the isoprenoid tail protons.[11] The largest signal of the spectrum is found at δ = 1.60 ppm as a singlet, which is assigned to the 7′, 11′, 15′, 19′, 23′, 27′ and 31′-methyl groups. The signal for the end (*trans*) 36-methyl group is found at δ = 1.68 ppm and the final (*cis*) 35′-methyl group is found at δ = 1.58 ppm. The 3′-tail methyl group closest to the quinone ring is found at δ = 1.62 ppm. The integral for this signal should be equal as that for the final (*trans*) 36-methyl group at δ = 1.68 ppm. If *cis*/*trans* isomerization occurs only the integral for the 3′-tail methyl group will decrease. Since for both of these integrals a value of 3 protons was found it could be concluded that the plastoquinone-9 was the pure *all-trans* isomer. At δ = 1.98 and 2.06 ppm the signals of all the methylene protons are found as two broad multiplets, except for the 1-methylene. This 1-methylene is found at δ = 3.12 ppm as a broad doublet with a coupling constant of J = 7.2 Hz with the 2-vinilic proton. It is also coupled with the 6-ring proton. The 2-vinylic proton is found at δ = 5.16 ppm as a broad triplet, since it has a long range coupling with the 6-ring proton. The 34-vinylic proton is found at δ = 5.09 ppm as a triplet. The other seven vinylic protons are found as a broad triplet at δ = 5.11 ppm.

The extra carbon−proton coupling in the proton spectra of the two labeled plastoquinones-9 (Figure 3) can be used to confirm the positions of the ^{13}C-labels. Since 3 bond coupling constants are bigger than 2 bond coupling constants, the ^{13}C-^{1}H-coupling constant for the 6-ring proton should be bigger for the 4-label than for the 1-label. In the spectrum of the 4-label a coupling constant of 9.9 Hz is observed and in that of the 1-label no coupling is observed. This confirms the position of the label. No extra coupling between any of the two ^{13}C-labels and the tail 2-methylene group protons could be observed.

With the extra carbon-proton coupling constants it is also possible to assign the two methyl groups unequivocally (Figure 3). The labels show a $^{3}J_{C-H}$ coupling with the closest methyl group signal of 3.7 Hz. The signals at δ = 2.01 and 2.03 ppm can be assigned to the 2′- and 3′-methyl groups, respectively.

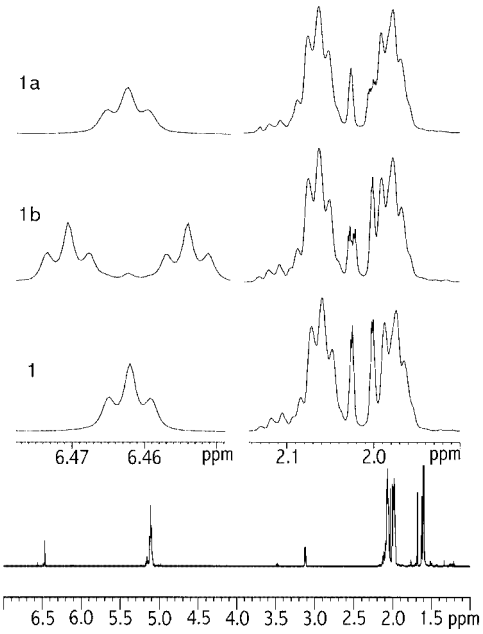


Figure 3. 600 MHz ^{1}H NMR spectra of plastoquinone-9 (bottom) and magnifications of the ring-proton (left) and ring-methyl signals (between the tail-methylene signals) (right) of 1-**^{13}**C- (1a), 4-**^{13}**C- (1b) and natural abundance- (1) plastoquinone-9.

The ring-proton signal would be the best signal to confirm the amount of ^{13}C-incorporation, since both methyl groups are already split to a doublet of quadruplets with a small coupling constant (< 1 Hz). For the 1-label no extra coupling is observed and therefore it is not possible to confirm the incorporation in this way. For the 4-label a small center signal is observed which is most likely due to a superpositioning of the overlapping triplet signals. This means that the degree of ^{13}C-enrichment as already found by mass spectrometry cannot be confirmed in this case by ^{1}H NMR spectroscopy.

^{13}C NMR Spectroscopy

To confirm the structure of the labeled products and the location of the ^{13}C-labels in the synthesized products ^{1}H-noise decoupled 150-MHz ^{13}C NMR of 1-^{13}C- and 4-^{13}C-6-bromoplastoquinone-0 and of 1-^{13}C- and 4-^{13}C-plastoquinone-9 were recorded. The spectra of the four labeled plastoquinones all show only one large signal: δ = 179.5 and 184.6 ppm for the 1-^{13}C- and 4-^{13}C-6-bromoplastoquinone-0 respectively and δ = 187.8 and 187.6 ppm for the 1-^{13}C- and 4-^{13}C-plastoquinone-9, respectively. This proves that only one position is labeled and that no scrambling has occurred during the synthesis.

The signal for the 2′- and 3′-methyl groups of bromoplastoquinone-0 are found at δ = 13.2 ppm and 12.3 ppm, respectively. The brominated C-6 gives a signal at δ = 136.8 ppm and the C-5 at δ = 137.7 ppm. C-2 and C-3 are assigned to the signals at δ = 140.8 and 141.2 ppm, respectively. The 1- and 4-carbonyl signals are found at δ = 179.5 and 184.6 ppm, respectively.

In the spectra of the labeled compounds, the remaining natural abundance carbon atoms 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 (Table 2). In the spectrum of 1-^{13}C-6-bromoplastoquinone-0 a typical one bond coupling constant of 51.6 Hz can be found, for example, between the 1 (labeled) carbonyl carbon and the 6-carbon atom. This confirms that the ^{13}C-labels are on the expected positions.

Table 2. Coupling constants (in Hz) of 1-^{13}C- and 4-^{13}C-6-bromoplastoquinone-0 and plastoquinone-9.

carbon	1	2	3	4	5	6	2′	3′	1-tail	2-tail
1-^{13}C-BrQ	–	52.9	2.1	7.3	2.4	51.6	1.5	4.1		
4-^{13}C-BrQ	7.3	2.0	51.5	–	50.9	9.7	4.1	1.8		
1-^{13}C-PQ	–	50.1	2.0	8.1	<1.5	53.3	1.5	3.4	4.4	
4-^{13}C-PQ	8.1	2.0	51.0	–	49.0	1.7	3.7		<1.5	2.3

Coupling constants were also found between the methyl carbon atoms and the labels on the 1- and 4-C atoms. Because 3 bond coupling constants are larger than 2 bond coupling constants, it is possible to distinguish between the two methyl groups. The 1-carbonyl, for example has a coupling constant of 1.5 and 4.1 Hz for its corresponding 2- and 3 bond coupling constants with the 2′- and 3′-methyl groups, respectively. The signals at δ = 13.2 and 12.3 ppm could therefore be unequivocally assigned to the 2′- and 3′-methyl groups, respectively.

The signals from the ring of plastoquinone-9 can be found at δ = 12.0 and 12.3 ppm for the 2′- and 3′-methyl groups. At δ = 132.0 ppm the signal is found for carbon 6 and at δ = 147.9 ppm for the 5-carbon. C-2 and C-3 can be assigned to the signals at δ = 140.5 and 140.9 ppm, respectively. The 1- and 4-carbonyl signals are found at δ = 187.8 and 187.6 ppm, respectively.

The remaining signals are assigned to the isoprenoid tail.[11] Its 3′-methyl group is found at δ = 16.1 ppm. The 7′-, 11′-, 15′-, 19′-, 23′-, 27′- and 31′-methyl groups are all found as a multiplet at δ = 16.0 ppm, followed at δ = 17.6 ppm with the end (*cis*) 35′methyl group and end *trans* 36-methyl group at δ = 25.7 ppm. The 1-methyne signal is found at δ = 27.4 ppm and the 32-methyne at δ = 26.5 ppm. Between δ = 26.6–26.7 ppm the signals are found for the 4-, 8-, 12-, 16-, 20-, 24- and 28-methyne carbon atoms. At δ = 39.7 ppm a multiplet for the 5-, 9-, 13-, 17-, 21-, 25-, 29-, and 33-methyne carbon atoms can be found. The 2-vinylic signal is found at δ = 118.1 ppm and the 6-vinylic signal at δ = 123.8 ppm. The remaining 10-, 14-, 18-, 22-, 26-, 30- and 34-CH vinylic signals are found between δ = 124.1–124.4 ppm as seven separate signals. The 3-quaternary carbon signal is found at δ = 139.6 ppm and the C-7 signal at δ = 135.4 ppm. The other 11-, 15-, 19-, 23-, 27-, 31-quaternary carbon atoms are found between δ = 134.8–135.0 ppm as separate signals, except for the final 35-carbon that has a signal at δ = 131.2 ppm.

In the spectra of the labeled compounds, the remaining natural abundance carbon atoms 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 (Table 2) and to confirm the assignment of the isoprenoid tail signals close to the ring. In the spectrum of 1-^{13}C-plastoquinone-0 a typical one bond coupling constant of 53.3 Hz can be found, for example, between the 1 (labeled) carbonyl carbon and the 6-carbon. This confirms that the ^{13}C-labels are on the expected positions.

Coupling constants were found of around 3.7 Hz between the labels on the 1- and 4-carbon atoms and one of the ring methyl-carbon atoms. Because coupling constants over three bonds are larger than two-bond coupling constants, the signals of these two methyl groups could be assigned to δ = 12.0 and 12.3 ppm for the 2′- and 3′-methyl groups, respectively.

Discussion

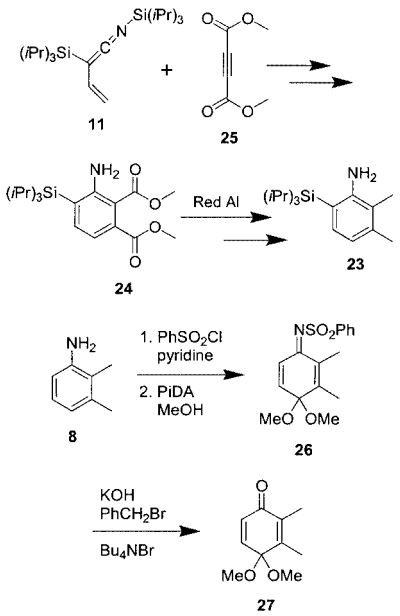
The 17-step synthetic scheme described in this paper gives access to 1-^{13}C- and 4-^{13}C-plastoquinones in reasonable overall yields of about 1% with levels of ^{13}C-incorporation above 98% and without isotope scrambling. The convergent synthetic scheme also makes it possible to label all single and any combination of positions in the quinone ring.

The intermediate ^{13}C-labeled products crotononitrile **12** and butynoate **13** are very useful building blocks for other ^{13}C-labeled syntheses. For instance crotononitrile **12** is used to synthesize specifically ^{13}C-labeled antranilic acid, which until now could not be labeled on all positions.[19] Since vitamin E, tocopherols and vitamin K resemble the structure of plastoquinone the synthetic schemes here provided can be used as a good starting point for their synthesis.

In order to develop the optimal way of incorporating ^{13}C-plastoquinones-9 into photosystem 2 sufficient amounts of plastoquinone-9 **1** are required, which is not commercially available and is laboursome to isolate. A two step sequence was deduced from the presented synthetic route, which was used to prepare 250 mg (30% overall yield) of plastoquinone-9 **1** from commercially available 2,3-dimethylphenol **7** by oxidation with Fremy's salt[20] and subsequent reaction with solanesyltributyltin **21**.[16]

The Diels–Alder reaction was initially performed with dimethyl acetylenedicarboxylate (**25**) in dichloromethane at −80 °C in eight hours in 54% yield (Scheme 4). The obtained dimethyl 3-amino-4-(triisopropylsilyl)phthalate (**24**) can be completely reduced to 2,3-dimethylaniline **23** by Red-Al in refluxing xylene in 16 hours with 50% yield. The yield (11%) of this alternative reaction sequence to 2,3-dimethylaniline (**8**) is slightly lower than the yield (16%) for the corresponding sequence with butynoate **13**. This alternative sequence cannot be used for specifically labeled

plastoquinones on the dimethyl side of the quinone ring since acetylenedicarboxylate **25** is a symmetric molecule.



Scheme 4. Alternative reaction schemes. PiDA = phenyliodonium diacetate

We recently found that intermediate **10** can be completely desilylated in one step with KF and 3 equivalents of HCl. The resulting 2-methoxycarbonyl-3-methylaniline is converted into aniline **8** with Red-Al. The overall yield of this shorter sequence is 14% higher than the one used for the labeled compounds.

Aniline **8** can be dibrominated to 4,6-dibromo-2,3-dimethylaniline without the risk of overbromination, contrary to the bromination reaction with phenol **7**. However 4,6-dibromo-2,3-dimethylaniline could not be oxidized to quinone **5** and could also not be converted into dibromophenol **6**. Therefore we had to convert aniline **8** to phenol **7** prior to bromination and subsequent oxidation with CAN to quinone **5**.

The new oxidation to quinone **5** with CAN gives yields of around 80%. It can also be performed with $Na_2Cr_2O_7{\cdot}2H_2O$ in a 1:1 mixture of 33% sulfuric acid in water and dichloromethane at 0 °C to room temp. in 20 hours with a yield of around 50%.

The MOM-protection is performed with chloromethyl methyl ether in extremely dry THF. We also tried to attach the MOM groups by using a mild acid catalyzed reaction with dimethoxymethane, which works perfectly on quinols without an isoprenoid tail. However, with isoprenylated quinols a tocopherol-like product was obtained. The protective MOM groups are not reactive towards strong bases like butyllithium and, most likely, stabilize the anion formed when di-MOM-ether **3** is treated with butyllithium. The di-MOM-protected quinols **2** and **3** can be excellently characterized with NMR spectroscopy, contrary to the also tried bis-triisopropylsilyl-protected quinols. These silyl protective groups also proved to be slightly reactive towards butyllithium. Finally the MOM groups can be easily oxidatively removed by CAN.

A "direct" attachment of the isoprenoid tail to the brominated carbon of quinone **5** would have been favorable. However a Stille reaction between quinone **5** and solanesyltributyltin compound **21** proved unsuccessful.[39] Instead of the expected plastoquinone-9 (**1**) only 5-butyl-2,3-dimethyl-*p*-benzoquinone was obtained. Unsuccessful was also a palladium-catalyzed reaction between solanesylzinc bromide and quinone **5**.[40]

An other approach to maintain asymmetry of the quinone ring, which would greatly reduce the amount of synthetic steps, was tried by preparing a specifically monoketalized plastoquinone-0 **27**. This monoketal can be easily prepared from aniline **8**, which is first transformed into a sulfonamide[41] followed by oxidation with phenyliodonium diacetate in methanol (Scheme 4).[42] The obtained quinone-imide ketal **26** is specifically saponificated to amide **26** in a two phase system with 1 N KOH with one equiv. of benzyl bromide to react with the intermediary formed nitrogen anion. Monoketal **27** is obtained in a yield of 77% (based on aniline **8**). A subsequent 1,4-addition reaction with the isoprenoid tail would deliver the plastoquinone-9 **1**. Unfortunately all tries to accomplish this last reaction stayed unsuccessful.

Conclusion

The syntheses here reported for plastoquinones labeled on all and any combination of positions has proved to be successful. The syntheses of 1-^{13}C- and 4-^{13}C-plastoquinone-9 have succeeded on a 100 milligram scale in reasonable yields of 1%. NMR spectroscopy and mass spectrometry proved that the incorporations were effected without scrambling or dilution of the ^{13}C-label. Also 250 mg (30% overall yield) plastoquinone-9 has been prepared by a two step synthesis. Furthermore two synthetic schemes for useful ^{13}C-labeled building blocks have been described for crotononitrile **12** and for triple bond containing compounds like methyl 2-butynoate (**13**).

Experimental Section

General: 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 the size 40−63 μm.

All reactions were performed in flame dried glassware under a dry nitrogen or argon atmosphere and with dry distilled solvents except when stated otherwise. Ether and PE were distilled from P_2O_5 and stored on sodium wire. THF and diisopropylamine were dried and stored on 4- Å molecular sieves. Methanol was distilled from magnesium curls and stored on 3- Å molecular sieves. Chlorotriisopropylsilane was always freshly distilled from CaH_2 prior to use.

NMR spectra were recorded on a Jeol NM FX-200 or on a Bruker DPX-300 instrument and a Bruker DMX600 instrument. All samples were dissolved in deuteriochloroform except when noted

otherwise. For ^{1}H NMR spectra either tetramethylsilane (δ = 0.00 ppm) or deuteriochloroform (δ = 7.26 ppm) was used as internal standards. Deuteriochloroform (δ = 77.0 ppm) was used as internal standard for the ^{13}C NMR spectra. The chemical shifts (δ) are given in ppm. In the NMR spectroscopic data of the ^{13}C-labeled compounds only the proton signals with an additional ^{13}C−H-coupling constant, the carbon signal of the ^{13}C-labeled atom and the 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.

Mass-spectrometry was performed on a Finnigan MAT 900 (electron impact, 70 eV) with PFK as a reference. The FT-IR spectra were recorded on a Perkin−Elmer FT-IR spectrometer, Paragon 1000.

For clarity the numbering for the carbon atoms are chosen in such a way that every carbon keeps the same number throughout the syntheses and therefore do often not follow the IUPAC rules.

Synthesis of 2-Butenenitrile (Crotononitrile)

3-Hydroxybutyronitrile (18): At −20 °C 7.45 mL (53.2 mmol) diisopropylamine was dissolved in 120 mL THF, followed by addition of 33 mL (52.8 mmol) of a 1.6 M butyllithium solution via a syringe. After 10 minutes this solution was cooled down to −80 °C and 2.70 mL (51.7 mmol) acetonitrile in 10 mL THF was added dropwise, followed after 15 minutes by 5.05 mL (51.7 mmol) ethyl acetate in 10 mL THF. This was stirred for 3 hours at −60 °C followed by addition of 1.20 g (31.7 mmol) $NaBH_4$. This mixture was stirred for 2 hours while it was allowed to warm up to room temperature. The suspension was then cooled with an icebath and 10 mL of concentrated hydrochloric acid in 10 mL water was added slowly under vigorous stirring. The mixture was subsequently stirred at 55 °C for 15 minutes to destroy the remaining boric salts. The solution was decanted and the residue was washed twice with ether. The combined organic phase was washed with 75 mL of a saturated NaCl solution. The (salty) water layer was subsequently extracted with ether. The combined organic phase was dried over $MgSO_4$. After evaporation under vacuum the crude product was purified by flash column chromatography with ether as eluens. This yielded 3.96 g (90%) of product. ^{1}H NMR (200 MHz): δ = 1.34 [d, ^3J(H−H) = 6.2 Hz, 3 H, CH_3], 2.50 [dd, ^2J(H−H) = 16.5 Hz and ^3J(H-H) = 6.0 Hz, 1 H, CH_AH$_B$], 2.58 [dd, ^2J(H-H) = 16.5 Hz and ^3J(H-H) = 5.2 Hz, 1 H, CH$_A$$H_B$], 3.24 [br. s, 1 H, OH], 4.15 [ddq, ^3J(H-H) = 6.2, ^3J(H-H) = 5.2, ^3J(H-H) = 6.0 Hz, 1 H, CH] ppm. ^{13}C NMR (50 MHz): δ = 22.5 (CH_3), 27.3 (CH_2), 63.7 (CH), 117.7 (CN) ppm.

[4-^{13}C]-3-Hydroxybutyronitrile (18b): The above described procedure was performed with 11.4 mL (81.3 mmol) diisopropylamine, 50 mL (80.0 mmol) of a 1.6 M butyllithium solution, 4.20 mL (89.1 mmol) acetonitrile, 7.00 g (78.6 mmol) ethyl 2-^{13}C-acetate, and 1.89 g (50.0 mmol) sodium borohydride. The yield was 4.59 g (68%). ^{1}H NMR (300 MHz): δ = 1.34 [dd, ^3J(H-H) = 6.2 Hz and ^1J(C-H) = 126.7 Hz, 3 H, $^{13}CH_3$], 2.50 [ddd, ^2J(H-H) = 16.5, ^3J(H-H) = 6.0 Hz and ^3J(C-H) = 3.3 Hz, 1 H, CH_AH$_B$], 2.58 [ddd, ^2J(H-H) = 16.5, ^3J(H-H) = 5.2 Hz and ^3J(C-H) = 3.7 Hz, 1 H, CH$_A$$H_B$] ppm. ^{13}C NMR (75 MHz): δ = 22.4 ($^{13}CH_3$), 63.7 [d, ^1J(C-C) = 38.8 Hz, CH] ppm.

3-(*p*-Tolylsulfonyloxy)butyronitrile: To 3.83 g (45.0 mmol) 3-hydroxybutyronitrile (**18**) dissolved in 30 mL dichloromethane was added 11.0 g (57.7 mmol) *p*-toluenesulfonyl chloride and 8 mL (98.9 mmol) pyridine. This solution was stirred for two days after which 50 mL ether and 10 mL water were added. The organic phase was separated and the water layer was extracted twice with ether. The combined organic layers were washed with a saturated Na_2CO_3 solution and a saturated NaCl solution and dried over $MgSO_4$. After evaporation under vacuum a crude yield of 10.97 g was obtained. The product was brought onto a silica column, where the excess *p*-toluenesulfonyl chloride was removed by elution with 20% ether/80% PE. The product was eluted with ether. After evaporation under vacuum a yellow liquid was obtained in a yield of 8.19 g (76%). ^{1}H NMR (200 MHz): δ = 1.43 [d, ^3J(H-H) = 6.5 Hz, 3 H, 4-CH_3], 2.46 (s, 3 H, tol-CH_3), 2.65 [dd, ^2J(H-H) = 17.0 Hz and ^3J(H-H) = 5.5 Hz, 1 H, CH_AH$_B$], 2.75 [dd, ^2J(H-H) = 17.0, ^3J(H-H) = 5.2 Hz, 1 H, CH$_A$$H_B$], 4.78 [ddq, ^3J(H-H) = 6.5, ^3J(H-H) = 5.2 Hz and ^3J(H-H) = 5.5 Hz, 1 H, 3-CH], 7.37 [d, ^3J(H-H) = 8.4 Hz, 2 H, 3′- and 5′-CH], 7.81 [d, ^3J(H-H) = 8.4 Hz, 2 H, 2′- and 6′-CH] ppm. ^{13}C NMR (50 MHz): δ = 20.0 (4-CH_3), 21.5 (tol-CH_3), 25.3 (CH_2), 72.8 (3-CH), 115.2 (CN), 127.6 (3′- and 5′-CH), 129.9 (2′- and 6′-CH), 133.0 (4′-C), 145.3 (1′-C) ppm.

[4-^{13}C]-3-(*p*-Tolylsulfonyloxy)butyronitrile: The above described procedure was performed with 4.59 g (53.3 mmol) [4-^{13}C]-3-hydroxybutyronitrile (**18b**) and 11.4 g (59.8 mmol) *p*-toluenesulfonyl chloride. The yield was 9.07 g (71%). ^{1}H NMR (300 MHz): δ = 1.43 [dd, ^3J(H-H) = 6.5 Hz and ^1J(H-H) = 128.9 Hz, 3 H, 4-$^{13}CH_3$], 2.65 [ddd, ^2J(H-H) = 17.0, ^3J(H-H) = 5.5 Hz and ^3J(C-H) = 4.2 Hz, 1 H, CH_AH$_B$], 2.75 [ddd, ^2J(H-H) = 17.0, ^3J(H-H) = 5.1 Hz and ^3J(C-H) = 3.2 Hz, 1 H, CH$_A$$H_B$] ppm. ^{13}C NMR (75 MHz): δ = 19.9 ($^{13}CH_3$), 72.8 [d, ^1J(C-C) = 39.3 Hz, 3-CH] ppm.

2-Butenenitrile (12). Method B: A 100-ml round-bottom flask filled with 8.19 g (34.2 mmol) 3-(*p*-tolylsulfonyloxy)butyronitrile and 4.9 g (39.5 mmol) 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) was fitted to two consecutive cold traps of witch the last one was cooled in liquid nitrogen. At 220 Torr this mixture was heated in 60 minutes to 150 °C where it was kept for 45 minutes. The product was found in both traps as a mixture of E/*Z*-2-butenenitrile (**12**) in a yield of 1.82 g (79%). ^{1}H NMR (300 MHz): *Z*-isomer: δ = 2.06 [dd, ^3J(H-H) = 7.0 Hz and ^4J(H-H) = 1.7 Hz, 3 H, CH_3], 5.35 [dq, ^3J(H-H) = 10.9 Hz and ^4J(H-H) = 1.7 Hz, 1 H, 2-CH], 6.58 [dq, ^3J(H-H) = 10.9 Hz and ^3J(H-H) = 7.0 Hz, 1 H, 3-CH] ppm. *E*-isomer: δ = 1.94 [dd, ^3J(H-H) = 6.9 Hz and ^4J(H-H) = 1.8 Hz, 3 H, CH_3], 5.36 [dq, ^3J(H-H) = 16.3 Hz and ^4J(H-H) = 1.8 Hz, 1 H, 2-CH], 6.74 [dq, ^3J(H-H) = 16.3 Hz and ^3J(H-H) = 6.9 Hz, 1 H, 3-CH] ppm. ^{13}C NMR (75 MHz): *Z*-isomer: δ = 17.4 (CH_3), 100.5 (2-CH), 115.8 (CN), 150.0 (3-CH) ppm. *E*-isomer: δ = 19.0 (CH_3), 100.8 (2-CH), 117.3 (CN), 151.2 (3-CH) ppm.

[4-^{13}C]-2-Butenenitrile (12b): The above described procedure was performed with 8.98 g (37.4 mmol) [4-^{13}C]-3-(*p*-tolylsulfonyloxy)-butyronitrile and 6.0 mL (48.6 mmol) DBN. The yield was 1.57 g (62%). ^{1}H NMR (600 MHz): *Z*-isomer: δ = 2.05 [ddd, ^3J(H-H) = 7.0, ^4J(H-H) = 1.7 Hz and ^1J(C-H) = 128.3 Hz, 3 H, $^{13}CH_3$], 5.35 [ddq, ^3J(H-H) = 10.9, ^4J(H-H) = 1.7 Hz and ^3J(C-H) = 10.9 Hz, 1 H, 2-CH], 6.58 [ddq, ^3J(H-H) = 10.9, ^3J(H-H) = 7.0 Hz and ^2J(C-H) = 3.4 Hz, 1 H, 3-CH] ppm. *E*-isomer: δ = 1.93 [ddd, ^3J(H-H) = 6.9, ^4J(H-H) = 1.8 Hz and ^1J(C-H) = 128.2 Hz, 3 H, $^{13}CH_3$], 5.36 [ddq, ^3J(H-H) = 16.3, ^4J(H-H) = 1.8 Hz and ^3J(C-H) = 7.6 Hz, 1 H, 2-CH], 6.74 [ddq, ^3J(H-H) = 16.3, ^3J(H-H) = 6.9 Hz and ^2J(C-H) = 3.4 Hz, 1 H, 3-CH] ppm. ^{13}C NMR (150 MHz): *Z*-isomer: δ = 17.4 ($^{13}CH_3$), 100.7 [d, ^2J(C-C) = 1.4 Hz, 2-CH], 115.9 [d, ^3J(C-C) = 4.3 Hz, CN], 150.0 [d, ^1J(C-C) = 41.5 Hz, 3-CH] ppm. *E*-isomer: δ = 19.0 ($^{13}CH_3$), 101.0 [d, ^2J(C-C) = 2.3 Hz, 2-CH], 117.4 [d, ^3J(C-C) = 8.2 Hz, CN], 151.2 [d, ^1J(C-C) = 41.6 Hz, 3-CH] ppm.

2-Butenenitrile (12). Method A: To 5.0 g (76.9 mmol) KCN dissolved in 12 mL H_2O were added 0.15 g KI and 1.93 g benzyltriethylammonium chloride. To this solution were added 33 mL dichloromethane and 17.0 mL allyl chloride. This reaction mixture was refluxed for 2 days followed by addition of 50 mL water. The water layer was separated and extracted twice with 20 mL dichloromethane. The combined organic layers were dried over $MgSO_4$ and the solvent was carefully removed by distillation over a vigreux equipped distillation setup. The remaining liquid was distilled in a mini-distillation setup. One fraction was collected at 75−110 °C giving 1.30 g of a liquid which contained E/*Z*-2-butenenitrile (**12**), allyl cyanide and dichloromethane (±60%). A second fraction was collected at 110−121 °C containing 2.64 g (51%) of pure E/*Z*-2-butenenitrile (**12**).

[1-^{13}C]-2-Butenenitrile (12a): The above described procedure was performed with 5.00 g (75.6 mmol) K^{13}CN, 2.03 g (8.9 mmol) benzyltriethylammonium chloride, 0.19 g (11.4 mmol) KI and 17.4 mL (213.5 mmol) allyl chloride. The product was collected at 90-^{13}0 °C with a yield of 2.77 g (54%). ^{1}H NMR (300 MHz; no coupling constants were observed between ^{13}CN and 2-CH): *Z*-isomer: δ = 2.06 [ddd, ^3J(H-H) = 7.0, ^4J(H-H) = 1.7 Hz and ^4J(C-H) = 1.8 Hz, 3 H, CH_3], 6.58 [ddq, ^3J(H-H) = 10.9, ^3J(H-H) = 7.0 Hz and ^3J(C-H) = 15.9 Hz, 1 H, 3-CH] ppm. *E*-isomer: δ = 1.94 [ddd, ^3J(H-H) = 6.9, ^4J(H-H) = 1.8 Hz and ^4J(C-H) = 1.5 Hz, 3 H, CH_3], 6.74 [ddq, ^3J(H-H) = 16.3, ^3J(H-H) = 6.9 Hz and ^3J(C-H) = 8.2 Hz, 1 H, 3-CH] ppm. ^{13}C NMR (75 MHz): *Z*-isomer: δ = 17.4 [d, ^3J(C-C) = 4.6 Hz, CH_3], 100.5 [d, ^1J(C-C) = 79.6 Hz, 2-CH], 115.8 (^{13}CN) ppm. *E*-isomer: δ = 19.0 [d, ^3J(C-C) = 8.3 Hz, CH_3], 100.8 [d, ^1J(C-C) = 77.4 Hz, 2-CH], 117.3 (^{13}CN) ppm.

Synthesis of Methyl 2-Butynoate

[(Methoxycarbonyl)methyl]triphenylphosphonium Bromide: To 18.0 g (68.6 mmol) triphenylphosphane in 120 mL ethyl acetate was added 6.50 mL (68.6 mmol) methyl bromoacetate (**16**) in 30 mL ethyl acetate. After stirring overnight the white precipitate was filtered off, washed with ether and dried under vacuum at 40 °C for 4 h. A yield of 27.6 g (97%) was obtained. ^{1}H NMR (200 MHz): δ =3.61 (s, 3 H, OCH_3), 5.62 [d, ^3J(P-H) = 15.5 Hz, 2 H, CH_2), 7.6−8.0 (m, 15 H, Phe) ppm.

[(Methoxycarbonyl)methylene]triphenylphosphorane (15): A separation funnel filled with 27.6 g (66.4 mmol) [(methoxycarbonyl)methyl]triphenylphosphonium bromide in 150 mL dichloromethane and 100 mL 1 M NaOH was vigorously shaken. The layers were separated and the water layer was extracted twice with dichloromethane. The combined organic layers were washed with a saturated NaCl solution and dried over $MgSO_4$. After evaporation under vacuum 22.1 g (99%) of a white solid was obtained. ^{1}H NMR (200 MHz): δ = 2.90 and 3.52 (1:3, 3 H, OCH_3), 7.4−7.8 (m, 15 H, Phe) ppm (CH not visible).

[Acetyl(methoxycarbonyl)methylene]triphenylphosphorane (14): To a suspension of 22.1 g (66.1 mmol) [(methoxycarbonyl)methylene]triphenylphosphorane (**15**) in 300 mL toluene was added dropwise 2.35 mL (33.1 mmol) acetyl chloride (**17**) in 5 mL toluene. This mixture was stirred overnight, followed by addition of 4.55 mL triethylamine (32.6 mmol) in 5 mL toluene and subsequent dropwise addition of 1.20 mL (16.5 mmol) acetyl chloride (**17**) in 5 mL toluene. The mixture was stirred for 3 hours before another 2.20 mL (16.5 mmol) triethylamine and 0.60 (8.3 mmol) acetyl chloride (**17**) were added as before. The mixture was again stirred for 3 hours before a final 1.10 mL (8.3 mmol) triethylamine and 0.30 mL (4.1 mmol) acetyl chloride (**17**) were added. The mixture was stirred overnight and then filtrated. The precipitate was washed thoroughly with toluene. The solvent was evaporated under vacuum to obtain a crude yield of 21.7 g as a yellow solid. ^{1}H NMR (200 MHz): δ = 2.46 (s, 3 H, CH_3), 3.16 (s, 3 H, OCH_3), 7.3−7.8 (m, 15 H, Phe) ppm.

Methyl 2-Butynoate (13): A 100-ml long-necked round-bottom flask filled with 21.7 g (57.5 mmol) [acetyl(methoxycarbonyl)methylene]triphenylphosphorane (**14**) was fitted to two consecutive cold traps of witch the last one was cooled in liquid nitrogen. The flask was slowly heated at 15 Torr with a heating mantle until the compound was completely molten. While the long neck was kept warm, the mixture was heated vigorously for the reaction to occur. The reaction was completed by heating the flask with a flame. The product was collected in the first trap and the yield was 4.02 g (71%). If the product contains triphenylphosphane oxide it can easily be purified by vacuum distillation (15 Torr). ^{1}H NMR (200 MHz): δ = 1.99 (s, 3 H, CH_3), 3.76 (s, 3 H, OCH_3) ppm. ^{13}C NMR (50 MHz): δ = 3.4 (CH_3), 52.3 (OCH_3), 72.0 (2-C), 85.4 (3-C), 153.9 (C=O) ppm.

Synthesis of 6-Bromo-2,3-dimethylbenzoquinone. 2,*N*-Bis(triisopropylsilyl)-1,3-butadiene Ketenimine (11): To 9.0 mL (64.2 mmol) diisopropylamine in 100 mL THF at −40 °C in an Argon atmosphere was injected 40 mL (64 mmol) of a 1.6 M solution of butyllithium in hexane via a syringe. After 5 minutes 15.8 mL (73.8 mmol) triisopropylsilyl chloride was added followed by dropwise addition of 2.45 mL (30.1 mmol) 2-butenenitrile (**12**). The reaction mixture was allowed to warm up to room temperature in 3 h. After evaporation under vacuum the resulting liquid was distilled (0.3 Torr). The yellow/orange fraction was collected at 110−180 °C with a crude yield of 7.72 g (67%). The crude product was used immediately in the next reaction. ^{1}H NMR (200 MHz): δ = 1.12 [m, 42 H, $Si(CH(CH_3)_2)_3$], 4.76 [dd, ^3J(H-H) = 10.0 Hz and ^2J(H-H) = 1.7 Hz, 1 H, 4-CH_ZH_E], 4.82 [dd, ^3J(H-H) = 16.8 Hz and ^2J(H-H) = 1.7 Hz, 1 H, 4-CH_ZH_E], 5.87 [dd, ^3J(H-H) = 10.0 Hz and ^3J(H-H) = 16.8 Hz, 1 H, 3-CH] ppm.

[1-^{13}C]-2,*N*-Bis(triisopropylsilyl)-1,3-butadiene Ketenimine (11a): The above described procedure was performed with 12.5 mL (89.2 mmol) diisopropylamine, 56 mL (89.6 mmol) of a 1.6 M butyllithium solution, 22.0 mL (102.8 mmol) triisopropylsilyl chloride and 2.72 g (40.0 mmol) [1-^{13}C]-2-butenenitrile (**12a**). After vacuum distillation the yield was 9.57 g (63%).

[4-^{13}C]-2,*N*-Bis(triisopropylsilyl)-1,3-butadiene Ketenimine (11b): The above described procedure was performed with 7.6 mL (54.2 mmol) diisopropylamine, 32 mL (51.2 mmol) of a 1.6 M butyllithium solution, 12.4 mL (57.9 mmol) triisopropylsilyl chloride and 1.57 g (23.1 mmol) [4-^{13}C]-2-butenenitrile (**12b**). After vacuum distillation the yield was 4.56 g (52%).

2-Methoxycarbonyl-3-methyl-6,*N*-bis(triisopropylsilyl)aniline (10): A Carius tube was filled with 7.72 g (20.3 mmol) 2,*N*-bis(triisopropylsilyl)-1,3-butadiene ketenimine (**11**), 2.05 mL (2.01 g, 20.5 mmol) methyl 2-butynoate (**13**) and 20 mL toluene under an argon atmosphere, after which it was cooled in liquid nitrogen and sealed under vacuum (±3 Torr). The tube was heated in a suitable oven at 160 °C for 65 hours. After cooling in liquid nitrogen the tube was opened and the solvent evaporated under vacuum. After purification over a silica column with dichloromethane as eluens a yield of 9.32 g [65% in regard to 2-butenenitrile (**12**)] was obtained. ^{1}H NMR (200 MHz): δ = 0.90−1.50 [m, 42 H, $Si(CH(CH_3)_2)_3$], 2.20 (s, 3 H, CH_3), 3.77 (s, 3 H, OCH_3), 5.29 (s, 1 H, NH), 6.65 [d, ^3J(H-H) = 7.2 Hz, 1 H, 4-CH], 7.25 [d, ^3J(H-H) = 7.2 Hz, 1 H, 5-CH] ppm.

[1-^{13}C]-2-Methoxycarbonyl-3-methyl-6,*N*-bis(triisopropylsilyl)-aniline (10a): The above described procedure was performed with 9.57 g (25.2 mmol) 2,*N*-bis(triisopropylsilyl)-1,3-butadiene ketenimine (**11a**) and 2.60 g [26.5 mmol) methyl-2-butynoate (**13**). The yield was 11.9 g (62% in regard to 2-butenenitrile **12a**). ^{13}C NMR (50 MHz): δ = 149.7 ($^{13}CNH_2$) ppm.

[4-^{13}C]-2-Methoxycarbonyl-3-methyl-6,*N*-bis(triisopropylsilyl)-aniline (10b): The above described procedure was performed with 4.56 g (12.0 mmol) 2,*N*-bis(triisopropylsilyl)-1,3-butadiene ketenimine (**11b**) and 1.60 g (16.3 mmol) methyl-2-butynoate (**13**). The yield was 5.3 g (48% in regard to[4-^{13}C]-2-butenenitrile (**12b**). ^{1}H NMR (200 MHz: δ = 2.20 [d, ^{3}J(C-H) = 4.8 Hz, 3 H, CH_3], 6.65 [dd, ^{3}J(H-H) = 7.2 Hz and ^{1}J(C-H) = 159.8 Hz, 1 H, ^{13}CH] ppm. ^{13}C NMR (50 MHz): δ = 120.7 (^{13}CH) ppm.

2-Methoxycarbonyl-3-methyl-6-(triisopropylsilyl)aniline (9): To 9.32 g (19.5 mmol) 6,*N*-bis(triisopropylsilyl)-2-methoxycarbonyl-3-methylaniline (**10**) in 50 mL methanol were added 4.2 g KF and 2.4 mL concd. HCl dissolved in 10 mL methanol. After 2 hours of refluxing the solvent was evaporated under vacuum and replaced by ether and water. The water layer was separated and extracted twice with ether. The combined organic layers were washed with a saturated NaCl solution and dried over $MgSO_4$. After evaporation under vacuum the crude product was purified over a column with dichloromethane as eluens yielding 3.89 g (62%) of product. ^{1}H NMR (200 MHz): δ = 1.10 [m, 18 H, Si[CH(CH_3)$_2$]$_3$], 1.40 [m, 3 H, Si[CH(CH_3)$_2$]$_3$], 2.38 (s, 3 H, CH_3), 3.87 (s, 3 H, OCH_3), 5.27 (s, 2 H, NH_2), 6.53 [d, ^{3}J(H-H) = 7.5 Hz, 1 H, 4-CH], 7.26 [d, ^{3}J(H-H) = 7.5 Hz, 1 H, 5-CH] ppm.

[1-^{13}C]-2-Methoxycarbonyl-3-methyl-6-(triisopropylsilyl)aniline (9a): The above described procedure was performed with 11.90 g (24.9 mmol) [1-^{13}C]-6,*N*-bis(triisopropylsilyl)-2-methoxycarbonyl-3-methylaniline (**10a**), 8.90 g KF and 5.2 mL concd. HCl. After purification with column chromatography 4.58 g (57%) product was obtained. ^{1}H NMR (300 MHz): δ = 7.26 [dd, ^{3}J(H-H) = 7.5 Hz and ^{3}J(C-H) = 10.4 Hz, 1 H, 5-CH] ppm. ^{13}C NMR (75 MHz): δ = 153.7 ($^{13}CNH_2$) ppm.

[4-^{13}C]-2-Methoxycarbonyl-3-methyl-6-(triisopropylsilyl)aniline (9b): The above described procedure was performed with 7.3 g (11.1 mmol) [4-^{13}C]-6,*N*-bis(triisopropylsilyl)-2-methoxycarbonyl-3-methylaniline (**10b**), 6.0 g KF and 3.0 mL concd. HCl. After purification with column chromatography 1.71 g (44%) product was obtained. ^{1}H NMR (300 MHz): δ = 2.38 (d, ^{3}J(C-H) = 6.0 Hz, 3 H, CH_3], 6.53 [dd, ^{3}J(H-H) = 7.5 Hz and ^{1}J(C-H) = 159.8 Hz, 1 H, ^{13}CH] ppm. ^{13}C NMR (75 MHz): δ = 119.8 (^{13}CH) ppm.

2,3-Dimethyl-6-(triisopropylsilyl)aniline: To 3.89 g (12.1 mmol) of 2-methoxycarbonyl-3-methyl-6-(triisopropylsilyl)aniline (**9**) in 150 mL toluene was added 28 mL of a 3.4 M Red-Al [sodium bis(2-methoxyethoxy)aluminum hydride] solution in toluene. After reflux for 3 hours 16.8 g of "wet"-silica (120 mL water absorbed in 400 g silica) was carefully added while cooled with an ice-bath and subsequently stirred for 30 minutes. Then $MgSO_4$ was added and the mixture was stirred for another 90 minutes. After filtration over Hyflo Super Cel and evaporation under vacuum 3.85 g of crude product was obtained. The product can be purified further with the additional purification procedure described for the 1-^{13}C-label. ^{1}H NMR (200 MHz): δ = 1.10 [m, 18 H, Si[CH(CH_3)$_2$]$_3$], 1.40 [m, 3 H, Si[CH(CH_3)$_2$]$_3$], 2.04 (s, 3 H, 2′-CH_3), 2.26 (s, 3 H, 3′-CH_3), 3.7 (br. s, 2 H, NH_2), 6.62 [d, ^{3}J(H-H) = 7.6 Hz, 1 H, 4-CH], 7.10 [d, ^{3}J(H-H) = 7.6 Hz, 1 H, 5-CH] ppm.

[1-^{13}C]-2,3-Dimethyl-6-(triisopropylsilyl)aniline: The above described procedure was performed with 4.48 g (13.8 mmol) [1-^{13}C]-2-methoxycarbonyl-3-methyl-6-(triisopropylsilyl)aniline (**9a**), 33 mL (112.2 mmol) of a 3.4 M Red Al solution in toluene 21 g 'wet silica'. In addition the crude product was dissolved in 10 mL ether and 100 mL 1 M HCl and stirred for 5 minutes. After addition of 200 mL 1 M NaOH the product was extracted thoroughly with ether. The combined organic layers were washed with a saturated NaCl solution and dried over $MgSO_4$. After evaporation under vacuum 3.20 g (83%) of crude product was obtained. ^{1}H NMR (300 MHz): δ = 2.07 [d, ^{3}J(C-H) = 3.9 Hz, 3 H, 2′-CH_3], 7.10 [dd, ^{3}J(H-H) = 7.6 Hz and ^{3}J(C-H) = 10.2 Hz, 1 H, 5-CH] ppm. ^{13}C NMR (75 MHz): δ = 150.2 (CNH_2) ppm.

[4-^{13}C]-2,3-Dimethyl-6-(triisopropylsilyl)aniline: The above described procedure was performed with 1.71 g (5.29 mmol) [4-^{13}C]-6-triisopropylsilyl-2-methoxycarbonyl-3-methylaniline (**9b**), 13 mL (44.2 mmol) of a 3.4 M Red Al solution in toluene and 10 g 'wet'-silica. In addition the crude product was treated as described for the 1-^{13}C-label. After evaporation under vacuum 1.37 g (93%) of crude product was obtained. ^{1}H NMR (300 MHz): δ = 2.27 [d, ^{3}J(C-H) = 4.9 Hz, 3 H, 3′-CH_3], 6.62 [dd, ^{3}J(H-H) = 7.6 Hz and ^{1}J(C-H) = 157.4 Hz, 1 H, ^{13}CH] ppm. ^{13}C NMR (75 MHz): δ = 120.0 (^{13}CH) ppm.

2-Methoxycarbonyl-3-methylaniline: To 0.36 g (0.73 mmol) -2-methoxycarbonyl-3-methyl-6,*N*-bis(triisopropylsilyl)aniline (**10**) in 2 mL of methanol was added 0.16 g (2.69 mmol) KF and a solution of 0.22 mL (2.69 mmol) concentrated HCl in 1 mL methanol. This mixture was refluxed for 2 hours before it was neutralized with a saturated $NaHCO_3$ solution to pH 7 and extracted three times with ether. The combined organic layers were washed with a saturated NaCl solution and dried over $MgSO_4$. After evacuation under vacuum the crude product was purified over a silica column with dichloromethane as eluens. A yield of 0.12 g (91%) was obtained as a light yellow oil. ^{1}H NMR (200 MHz): δ = 1.33 [t, 3 H, ^{3}J(H-H) = 7.2 Hz, CH_3], 2.41 (s, 3 H, 3′-CH_3), 4.32 [q, 2 H, ^{3}J(H-H) = 7.2 Hz, OCH_2], 5.12 (br. s, 2 H, NH_2), 6.4−6.5 (m, 2 H, 4-CH and 6-CH), 7.01 [t, 1 H, ^{3}J(H-H) = 7.9 Hz, 5-H] ppm. ^{13}C NMR (50 MHz): δ = 14.0 (3′-CH_3), 22.7 (CH_3), 60.2 (CH_2O), 113.8 (2-C), 114.2 (6-C), 119.9 (4-C), 131.5 (5-C), 139.6 (3-C), 148.8 (1-C), 168.9 (s, CO) ppm.

2,3-Dimethylaniline (8). Procedure A: To 3.85 g of 2,3-dimethyl-6-triisopropylsilylaniline in 210 mL hexane was added 6.7 mL (87.0 mmol) trifluoroacetic acid. After reflux for 5 hours the mixture was cooled to room temperature and 80 mL 1 M NaOH was added. After 15 minutes of stirring, the water layer was separated and extracted twice with ether. The combined organic layers were dried over $MgSO_4$ and the solvents evaporated under vacuum. The crude product was dissolved in THF and 80 mL 1 M NaOH was added and stirred for 5 hours. The water layer was separated and extracted twice with ether. The combined organic layers were washed with a saturated NaCl solution and dried over $MgSO_4$. After evaporation under vacuum the crude product was purified over a silica column with a 30% ether/70% PE mixture as eluens. The yield was 0.65 g (44% in regard to 2-methoxycarbonyl-3-methyl-6-(triisopropylsilyl)aniline (**9**) of pure product.

Procedure B: To 0.62 g (3.44 mmol) 2-methoxycarbonyl-3-methylaniline in 45 mL dry toluene was added 7.9 mL (27.54 mmol) of a 3.4 M Red-Al solution in toluene. After refluxed for 3 hours 13.2 g 'wet' silica was carefully added while cooled with an ice-bath. After 30 min. Then $MgSO_4$ was added and stirring was continued for 1.5 hour. After filtration over Hyflo Super Cel and evaporation under

vacuum the crude product was purified over a silica column with 50% ether/50% PE as eluens. This yielded 0.31 g (75%) of product as orange oil. ^{1}H NMR (200 MHz): δ = 2.03 (s, 3 H, 2′-CH_3), 2.24 (s, 3 H, 3′-CH_3), 3.50 (br. s, 2 H, NH_2), 6.51 [d, ^3J(H-H) = 7.9 Hz, 1 H, 6-CH], 6.60 [d, ^3J(H-H) = 7.6 Hz, 1 H, 4-CH], 6.90 [dd, ^3J(H-H) = 7.9 Hz and ^3J(H-H) = 7.6 Hz, 1 H, 5-CH] ppm. ^{13}C NMR (50 MHz: δ = 12.5 (2′-CH_3), 20.3 (3′-CH_3), 113.0 (6-CH), 120.4 (4-CH), 120.6 (2-C), 125.8 (5-CH), 136.9 (3-C), 144.3 (CNH_2) ppm.

[1-^{13}C]-2,3-Dimethylaniline (8a): The above described procedure under A was performed with 1.34 g (4.8 mmol) of [1-^{13}C]-2,3-dimethyl-6-(triisopropylsilyl)aniline in 90 mL hexane and 2.6 mL (72.7 mmol) trifluoroacetic acid. After purification by column chromatography 0.50 g (29% in regard to [1-^{13}C]-2-methoxycarbonyl-3-methyl-6-(triisopropylsilyl)aniline (**9a**)] of pure product was obtained. ^{1}H NMR (300 MHz): δ = 2.03 [d, ^3J(C-H) = 4.0 Hz, 3 H, 2′-CH_3], 6.90 [ddd, ^3J(H-H) = 7.9, ^3J(H-H) = 7.6 Hz and ^3J(C-H) = 8 Hz, 1 H, 5-CH] ppm. ^{13}C NMR (75 MHz: δ = 12.6 [d, ^2J(C-C) = 0.9 Hz, 2′-CH_3], 20.4 [d, ^3J(C-C) = 3.3 Hz, 3′-CH_3], 113.1 [d, ^1J(C-C) = 62.4 Hz, 6-CH], 120.6 [d, ^3J(C-C) = 8.2 Hz, 4-CH], 120.9 [d, ^1J(C-C) = 62.4 Hz, 2-C], 137.1 [d, ^2J(C-C) = 1.4 Hz, 3-C], 144.4 ($^{13}CNH_2$) ppm.

[4-^{13}C]-2,3-Dimethylaniline (8b): The above described procedure under A was performed with 3.20 g (11.5 mmol) [4-^{13}C]-2,3-dimethyl-6-(triisopropylsilyl)aniline in 175 mL hexane and 5.6 mL (72.7 mmol) trifluoroacetic acid. After purification by column chromatography 0.23 g (35% in regard to 2-methoxycarbonyl-3-methyl-6-(triisopropylsilyl)aniline (**9b**)] of pure product was obtained. ^{1}H NMR (300 MHz): δ = 2.25 [d, ^3J(C-H) = 5.0 Hz, 3 H, 3′-CH_3], 6.53 [dd, ^3J(H-H) = 7.9 Hz and ^3J(C-H) = 7 Hz, 1 H, 6-CH], 6.61 [dd, ^3J(H-H) = 7.5 Hz and ^1J(C-H) = 159.5 Hz, 1 H, ^{13}CH], 6.90 [ddd, ^3J(H-H) = 7.5, ^3J(H-H) = 7.9 Hz and ^2J(C-H) = 2 Hz, 1 H, 5-CH] ppm. ^{13}C NMR (75 MHz: δ = 12.6 [d, ^3J(C-C) = 2.7 Hz, 2′-CH_3], 113.1 [d, ^2J(C-C) = 2.5 Hz, 6-CH], 120.6 (^{13}CH), 120.9 [d, ^2J(C-C) = 2.3 Hz, 2-C], 126.0 [d, ^1J(C-C) = 58.1 Hz, 5-CH], 137.0 [d, ^1J(C-C) = 58.0 Hz, 3-C], 144.4 [d, ^3J(C-C) = 8.3 Hz, CNH_2] ppm.

2,3-Dimethylphenol (7): To 0.65 g (5.33 mmol) of 2,3-dimethylaniline (**8**) was added 6.5 mL 35% H_2SO_4 and cooled with an ice-bath. To this mixture an ice-cold solution of 0.78 g (11.3 mmol) $NaNO_2$ in 10 mL water was pipetted slowly under its liquid surface. After 5 minutes of careful slow stirring 0.35 g (5.1 mmol) urea was added. After another 5 minutes a solution of 36 g $CuSO_4 \cdot 5H_2O$ in 290 mL H_2O was quickly added, the ice-bath was removed and under vigorous stirring 1.45 g Cu_2O was added. After stirring for 45 minutes the mixture was extracted three times with 75 mL ether. The combined organic layers were washed with a saturated NaCl solution and dried over $MgSO_4$. After evaporation under vacuum a crude yield of 0.49 g (77%) was obtained. ^{1}H NMR (200 MHz): δ = 2.16 (s, 3 H, 2′-CH_3), 2.27 (s, 3 H, 3′-CH_3), 4.70 (br. s, 1 H, OH), 6.63 [d, ^3J(H-H) = 7.5 Hz, 1 H, 6-CH], 6.75 [d, ^3J(H-H) = 7.5 Hz, 1 H, 4-CH], 6.96 [dd, ^3J(H-H) = 7.5 Hz and ^3J(H-H) = 7.5 Hz, 1 H, 5-CH] ppm. ^{13}C NMR (50 MHz: δ = 11.0 (2′-CH_3), 19.6 (3′-CH_3), 112.3 (6-CH), 122.0 (4-CH), 122.2 (2-C), 125.5 (5-CH), 137.9 (3-C), 152.9 (COH) ppm

[1-^{13}C]-2,3-Dimethylphenol (7a): The above described procedure was performed with 0.46 g (3.77 mmol) of 2,3-dimethylaniline (**8a**), 4.3 mL 35% H_2SO_4, 0.54 g (7.8 mmol) $NaNO_2$, 0.1 g (0.17 mmol) urea, 21.7 g (86.8 mmol) $CuSO_4 \cdot 5H_2O$ in 200 mL H_2O and 0.80 g (5.6 mmol) Cu_2O. The crude yield was 0.39 g (84%). ^{1}H NMR (300 MHz): δ = 2.15 [d, ^3J(C-H) = 6.1 Hz, 3 H, 2′-CH_3], 6.96 [ddd, ^3J(H-H) = 7.6, ^3J(H-H) = 7.6 Hz and ^3J(C-H) = 9.5 Hz, 1 H, 5-CH] ppm. ^{13}C NMR (75 MHz: δ = 20.0 [d, ^3J(C-C) = 3.8 Hz, 3′-CH_3], 112.5 [d, ^1J(C-C) = 66.0 Hz, 6-CH], 122.3 [d, ^3J(C-C) = 8.6 Hz 4-CH], 122.5 [d, ^1J(C-C) = 68.0 Hz, 2-C], 153.5 (^{13}COH) ppm.

[4-^{13}C]-2,3-Dimethylphenol (7b): The above described procedure was performed with 0.23 g (1.9 mmol) of 2,3-dimethylaniline (**8b**), 2.2 mL 35% H_2SO_4, 0.27 g (4.0 mmol) $NaNO_2$, 0.1 g (1.7 mmol) urea, 11.2 g (44.6 mmol) $CuSO_4 \cdot 5H_2O$ in 90 mL H_2O and 0.42 g (2.9 mmol) Cu_2O. The crude yield was 0.22 g (97%). ^{1}H NMR (300 MHz): δ = 2.27 [d, ^3J(C-H) = 5.0 Hz, 3 H, 3′-CH_3], 6.61 [dd, ^3J(H-H) = 7.6 Hz and ^3J(C-H) = 7.4 Hz, 1 H, 6-CH], 6.74 [dd, ^3J(H-H) = 7.6 Hz and ^1J(C-H) = 159.5 Hz, 1 H, ^{13}CH] ppm. ^{13}C NMR (75 MHz): δ = 11.4 [d, ^3J(C-C) = 2.7 Hz, 2′-CH_3], 112.6 [d, ^2J(C-C) = 2.6 Hz, 6-CH], 122.3 (^{13}CH), 122.6 [d, ^2J(C-C) = 3.1 Hz, 2-C], 126.0 [d, ^1J(C-C) = 56.2 Hz, 5-CH], 137.9 [d, ^1J(C-C) = 58.0 Hz, 3-C], 152.9 [d, ^3J(C-C) = 8.6 Hz, COH] ppm.

4,6-Dibromo-2,3-dimethylphenol (6): To 0.49 g (4.09 mmol) of 2,3-dimethylphenol (**7**) in 100 mL dichloromethane was added 0.05 g (0.9 mmol) iron powder. Under exclusion of light and at 0 °C 0.52 mL (1.61 g, 10.1 mmol) bromine in 5 mL dichloromethane was added dropwise. The mixture was stirred overnight at room temp., after which 20 mL H_2O, 10 mL saturated $Na_2S_2O_3$ and 10 mL 1 M HCl were added. The water layer was extracted twice with dichloromethane. The combined organic layers were washed with a saturated NaCl solution. After drying over $MgSO_4$ and evaporation under vacuum a crude yield of 0.96 g (84%) was obtained. ^{1}H NMR (300 MHz): δ = 2.27 (s, 3 H, 2′-CH_3), 2.31 (s, 3 H, 3′-CH_3), 5.50 (br. s, 1 H, OH), 7.50 (s, 1 H, 5-CH) ppm. ^{13}C NMR (75 MHz: δ = 14.1 (2′-CH_3), 19.7 (3′-CH_3), 107.5 (6-CBr), 115.7 (4-CBr), 125.7 (2-C), 131.2 (CH), 137.1 (3-C), 149.4 (COH) ppm.

[1-^{13}C]-4,6-Dibromo-2,3-dimethylphenol (6a): The above described procedure was performed with 0.38 g (3.0 mmol) of [1-^{13}C]-2,3-dimethylphenol (**7a**), 0.04 g (3.2 mmol) Fe powder and 0.4 mL (7.76 mmol) Br_2. The crude yield was 0.72 g of a mixture of [1-^{13}C]-4,6-dibromo-2,3-dimethylphenol (**7a**) (± 0.44 g, 52%) and [1-^{13}C]-4,5,6-tribromo-2,3-dimethylphenol (± 0.28 g). ^{1}H NMR (300 MHz): δ = 2.25 [d, ^3J(C-H) = 4.1 Hz, 3 H, 2′-CH_3], 7.47 [d, ^3J(C-H) = 7.9 Hz, 1 H, CH] ppm. ^{13}C NMR (75 MHz): δ = 19.7 [d, ^3J(C-C) = 3.5 Hz, 3′-CH_3], 107.5 [d, ^1J(C-C) = 70.8 Hz, 6-CBr], 115.7 [d, ^3J(C-C) = 7.2 Hz, 4-CBr], 125.7 [d, ^1J(C-C) = 70.1 Hz, 2-C], 131.2 [d, ^2J(C-C) = 2.2 Hz, CH], 137.1 [d, ^2J(C-C) = 2.0 Hz, 3-C], 149.4 (^{13}COH) ppm.

[4-^{13}C]-4,6-Dibromo-2,3-dimethylphenol (6b): The above described procedure was performed with 0.22 g (1.8 mmol) of [4-^{13}C]-2,3-dimethylphenol (**7b**), 0.01 g Fe powder and 0.15 mL (2.9 mmol) Br_2. The crude yield was 0.40 g (78%) ppm. ^{1}H NMR (300 MHz): δ = 2.31 [d, ^3J(C-H) = 5.7 Hz, 3 H, 3′-CH_3], 7.50 [d, ^2J(C-H) = 4.5 Hz, 1 H, CH] ppm. ^{13}C NMR (75 MHz: δ = 14.0 [d, ^3J(C-C) = 3.8 Hz, 2′-CH_3], 107.5 [d, ^2J(C-C) = 1.7 Hz, 6-CBr], 115.7 (^{13}CBr), 125.7 [d, ^2J(C-C) = 1.4 Hz, 2-C], 131.2 [d, ^1J(C-C) = 65.7 Hz, CH], 137.1 [d, ^1J(C-C) = 65.9 Hz, 3-C], 149.4 [d, ^3J(C-C) = 7.1 Hz, COH] ppm.

6-Bromo-2,3-dimethyl-*p*-benzoquinone (5): To 0.96 g (3.41 mmol) 4,6-dibromo-2,3-dimethylphenol (**6**) in 30 mL acetonitrile and 30 mL H_2O was added 2.90 g (17.4 mmol) 2,6-pyridinedicarboxylic acid and 7.61 g (13.9 mmol) ammonium cerium(VI) nitrate. After overnight stirring a saturated $NaHCO_3$ solution was added until the mixture reached a pH of 8. The product was extracted with ether. The combined organic phase was washed with a saturated NaCl solution and dried over $MgSO_4$. After evaporation under vacuum the crude product was purified over silica with 15% ether/

85% PE as eluens. The yield was 0.37 g (51%; m.p. 38−42 °C). The overall yield from 2-butenenitrile (**12**) is 6%. ^{1}H NMR (300 MHz): δ = 2.05 [q, ^5J(H-H) = 1.2 Hz, 3 H, 3′-CH_3], 2.11 [q, ^5J(H-H) = 1.2 Hz, 3 H, 2′-CH_3], 7.25 (s, 1 H, CH) ppm. ^{13}C NMR (75 MHz): δ = 12.4 (3′-CH_3), 13.3 (2′-CH_3), 136.9 (CBr), 137.8 (CH), 140.9 (2-C), 141.2 (3-C), 179.5 (1-CO), 184.6 (4-CO) ppm. MS (EI): *m*/*z* (rel. intensity) = 216 (23) [M$^+$], 214 (22) [M$^+$], 188 (25), 186 (29), 162 (12), 160 (15), 134 (33), 132 (34), 107 (100), 79(33), 77(67). HRMS (double focus, reference: PFK) calcd. for $C_8H_7{}^{79}BrO_2$: 213.9630, found 213.9647. FT-IR: ṽ = 3057 (m), 1664 (vs), 1645 (s), 1589 (s), 1378 (m), 1297 (m), 1237 (s), 1120 (s), 1080 (m), 910 (s), 845 (s), 785 (s), 685 (s), 670 (s), 498 (m), 408 (s) cm^{-1}.

[1-^{13}C]-6-Bromo-2,3-dimethyl-*p*-benzoquinone (5a): The above described procedure was performed with 0.68 g (2.4 mmol) of the mixture of [1-^{13}C]-4,6-dibromo-2,3-dimethylphenol (**6a**) and [1-^{13}C]-4,5,6-tribromo-2,3-dimethylphenol in 25 mL acetonitrile and 25 mL water, 2.03 g (12.2 mmol) 2,6-pyridinedicarboxylic acid and 5.31 g (9.7 mmol) ammonium cerium(VI) nitrate. Purification by column chromatography yielded 0.20 g of a mixture of [1-^{13}C]-6-bromo-2,3-dimethyl-1,4-benzoquinone (**5a**) (± 0.12 g, 36%) and [1-^{13}C]-5,6-dibromo-2,3-dimethyl-1,4-benzoquinone. ^{1}H NMR (600 MHz): δ = 2.05 [dq, ^5J(H-H) = 1.2 Hz and ^4J(C-H) = 1.2 Hz, 3 H, 3′-CH_3], 2.11 [dq, ^5J(H-H) = 1.2 Hz and ^3J(C-H) = 4.0 Hz, 3 H, 2′-CH_3], 7.25 [d, ^3J(C-H) = 8.3 Hz, 1 H, CH] ppm. ^{13}C NMR (150 MHz): δ = 12.4 [d, ^3J(C-C) = 4.1 Hz, 3′-CH_3], 13.3 [d, ^2J(C-C) = 1.5 Hz, 2′-CH_3], 136.9 [d, ^1J(C-C) = 51.6 Hz, CBr], 137.8 [d, ^2J(C-C) = 2.4 Hz, CH], 140.9 [d, ^1J(C-C) = 52.9 Hz, 2-C], 141.2 [d, ^2J(C-C) = 2.1 Hz, 3-C], 179.5 (^{13}CO), 184.6 [d, ^3J(C-C) = 7.1 Hz, 4-CO] ppm. MS *m*/*z* (rel. intensity) = 217 (100) [M$^+$], 215 (98) [M$^+$], 189 (16), 188 (32), 187 (19), 186 (33), 163 (6), 161 (8), 134 (17), 132 (15), 108 (21), 107 (35), 79 (27), 77 (27). HRMS (double focus) calcd. for $^{13}C_1{}^{12}C_7H_7{}^{79}BrO_2$: 214.9663, found 214.9652. FT-IR: ṽ = 2922 (m), 1653 (vs), 1618 (s), 1576 (s), 1558 (m), 1371 (m), 1248 (m), 1232 (m), 1140 (vs), 1120 (m) 1076 (m), 882 (s), 842 (m), 820 (m), 786 (m), 786 (m), 749 (m), 690 (s), 674 (s), 490 (m), 420 (s) cm^{-1}.

[4-^{13}C]-6-Bromo-2,3-dimethyl-*p*-benzoquinone (5b): The above described procedure was performed with 0.40 g (1.4 mmol) [4-^{13}C]-4,6-dibromo-2,3-dimethylphenol (**6b**) in 12 mL acetonitrile and 12 mL water, 1.21 g (7.2 mmol) 2,6-pyridinedicarboxylic acid and 3.19 g (5.8 mmol) ammonium cerium(VI) nitrate. After purification by column chromatography a yield of 0.17 g (55%) was obtained. ^{1}H NMR (300 MHz): δ = 2.05 [dq, ^5J(H-H) = 1.2 Hz and ^3J(C-H) = 3.9 Hz, 3 H, 3′-CH_3], 2.11 [dq, ^5J(H-H) = 1.2 Hz and ^4J(C-H) = 1.2 Hz, 3 H, 2′-CH_3], 7.25 [d, ^2J(C-H) = 0.7 Hz, 1 H, 4-CH] ppm. ^{13}C NMR (75 MHz): 13.3 [d, ^3J(C-C) = 4.1 Hz, 2′-C], 136.9 [d, ^2J(C-C) = 1.8 Hz, CBr], 137.8 [d, ^1J(C-C) = 50.9 Hz, CH], 140.9 [d, ^2J(C-C) = 2.0 Hz, 2-C], 141.2 [d, ^1J(C-C) = 51.5 Hz, 3-C], 179.5 [d, ^3J(C-C) = 7.5 Hz, 1-CO], 184.6 (^{13}CO) ppm. MS *m*/*z* (rel. intensity) = 217 (100) [M$^+$], 215 (96) [M$^+$], 189 (43), 188 (26), 187 (48), 186 (24), 163 (10), 161 (8), 135 (29), 133 (29), 108 (82), 107 (43), 79(25), 77(34). HRMS (double focus) calcd. for $^{13}C_1{}^{12}C_7H_7{}^{79}BrO_2$: 214.9663, found 214.9660. FT-IR: ṽ = 3057 (m), 1664 (vs), 1607 (s), 1592 (s), 1558 (m), 1377 (m), 1287 (m), 1231 (m), 1116 (vs), 1078 (m), 908 (s), 844 (m), 788 (m), 780 (m), 673 (s), 494 (m), 408 (s) cm^{-1}.

Synthesis of Plastoquinone-9

Solanesyl Bromide

(*all-E*)-3,7,11,15,19,23,27,31,35-Nonamethyl-2,6,10,14,18,22,26,30,34-hexatriacontanonaenyl Bromide: In 25 mL ether and 16 mL PE was dissolved 2.00 g (3.18 mmol) solanesol (**22**) and 5 drops pyridine. This mixture was cooled in an icebath before 0.24 mL (2.55 mmol) phosphorus tribromide was added dropwise. The mixture was stirred at 0 °C for 1 hour followed by 1 hour at room temperature before the mixture was carefully poured out into a separatory funnel partially filled with water, ice and PE. The water layer was separated and extracted three times with PE. The combined organic layer was carefully dried over $MgSO_4$ and the solvent was evaporated under vacuum. A crude yield of 2.18 g (99%) was obtained. ^{1}H NMR: δ = 1.60 (s, 24 H, 8 CH_3), 1.68 (s, 3 H, 36-CH_3), 1.73 (s, 3 H, 3′-CH_3), 2.01 (m, 32 H, 16 CH_2), 4.02 [d, ^3J(H-H) = 8.2 Hz, 2 H, CH_2Br], 5.1 [br. t, ^3J(H-H) = 7 Hz, 8 H, 8 CH], 5.53 [t, ^3J(H-H) = 8.2 Hz, 1 H, 2-CH] ppm.

Solanesyltributyltin (21)

(*all-E*)-3,7,11,15,19,23,27,31,35-Nonamethyl-2,6,10,14,18,22,26,30,34-hexatriacontanonaenyltributyltin: To 1.00 mL (7.14 mmol) diisopropylamine in 10 mL THF cooled to 0 °C was injected 3.90 mL (6.24 mmol) of a 1.6 M solution of butyllithium in hexane via a syringe. After 5 minutes 1.02 mL (3.78 mmol) tributyltin hydride was added. This mixture was stirred for 15 minutes and subsequently cooled to −60 °C before 2.18 g (3.15 mmol) solanesyl bromide in 20 mL THF was added dropwise. The mixture was allowed to warm up to −40 °C in 2 hours before 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 over $MgSO_4$. After evaporation of the solvent under vacuum a crude yield of 2.93 g 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, 24 H, 8 CH_3), 1.68 (s, 6 H, 3′-CH_3 and *cis* 35′-CH_3), 2.00 (m, 32 H, 16 CH_2), 5.1 [br. t, ^3J(H-H) = 7 Hz, 8 H, 8 CH], 5.32 [t, ^3J(H-H) = 9.3 Hz, 1 H, 3-CH] ppm.

6-Bromo-2,3-dimethyl-5-solanesyl-*p*-benzoquinone (4)

6-Bromo-2,3-dimethyl-5-[(*all-E*)-3,7,11,15,19,23,27,31,35-nonamethyl-2,6,10,14,18,22,26,30,34-hexatriacontanonaenyl]-*p*-benzoquinone: To 0.29 g (1.35 mmol) 6-bromo-2,3-dimethyl-*p*-benzoquinone (**5**) in 20 mL dichloromethane was added 1.25 g (5.39 mmol) silver oxide. This mixture was kept in the dark and was cooled to −80 °C, where 2.32 mL (18.86 mmol) of boron trifluoride−diethyl ether freshly distilled from CaH_2 was injected via a syringe. Then 2.46 g (2.73 mmol) solanesyltributyltin (**21**) in 30 mL dichloromethane was added dropwise. This mixture was allowed to warm up to −20 °C in 2 hours and was then cooled down to −40 °C before 20 mL of saturated NaCl solution was added and the cooling removed. The mixture was filtrated over Hyflo Super Cel. The water layer was separated and extracted twice with dichloromethane. The combined organic layer was dried with $MgSO_4$ and the solvents evaporated under vacuum. A crude yield of 1.77 g was obtained, which was dissolved in 20 mL THF before 20 mL water and 4 g potassium fluoride was added. After subsequent stirring for 30 minutes the water layer was separated and extracted twice with dichloromethane. The combined organic layer was washed with saturated NaCl solution and was subsequently dried over $MgSO_4$. Evaporated under vacuum yielded 1.44 g of crude product. Purification over silica with an eluens of 15% ether/85% PE yielded 0.45 g (0.54 mmol, 40%) of pure product and 0.17 g (59%) of pure 6-bromo-2,3-dimethyl-*p*-benzoquinone (**5**). ^{1}H NMR: δ = 1.56 (s, 3 H, tail 35′-CH_3), 1.60 (s, 21 H, tail 7 CH_3), 1.68 (s, 3 H, tail 36-CH_3), 1.78 (s, 3 H, tail 3′-CH_3), 2.01 (m, 38 H, tail 16 CH_2 and

ring 2 CH_3), 3.41 [d, ^3J(H-H) = 7.2 Hz, 2 H, tail 1-CH_2], 5.11 (m, 9 H, tail 9 CH) ppm.

[1-^{13}C]-6-Bromo-2,3-dimethyl-5-solanesyl-*p*-benzoquinone (4a): The above described procedure was performed with 0.20 g of a mixture of [1-^{13}C]-6-bromo-2,3-dimethyl-*p*-benzoquinone (**5a**) (± 0.12 g; 0.56 mmol) and [1-^{13}C]-5,6-dibromo-2,3-dimethyl-1,4-benzoquinone in 10 mL dichloromethane, 0.54 g (2.33 mmol) silver oxide, 0.97 mL (7.88 mmol) of freshly distilled boron trifluoride−diethyl ether, 1.00 g (1.11 mmol) solanesyltributyltin (**21**) in 15 mL dichloromethane and 4 g KF to obtain a crude yield of 1.14 g. After purification over silica, 0.16 g (34%) [1-^{13}C]-6-bromo-2,3-dimethyl-5-solanesyl-*p*-benzoquinone (**4a**) was obtained and 0.03 g (25%) pure of [1-^{13}C]-6-bromo-2,3-dimethyl-*p*-benzoquinone (**5a**) was recovered. ^{13}C NMR: δ = 179.8 (1-^{13}C=O) ppm.

[4-^{13}C]-6-Bromo-2,3-dimethyl-5-solanesyl-*p*-benzoquinone (4b): The above described procedure was performed with 0.17 g (0.79 mmol) [4-^{13}C]-6-bromo-2,3-dimethyl-*p*-benzoquinone (**5b**) in 10 mL dichloromethane, 0.54 g (2.33 mmol) silver oxide, 0.97 mL (7.88 mmol) of freshly distilled boron trifluoride−diethyl ether, 1.00 g (1.11 mmol) solanesyltributyltin (**21**) in 15 mL dichloromethane and 4 g KF to obtain a crude yield of 1.15 g. After purification over silica, 0.20 g (31%) [4-^{13}C]-6-bromo-2,3-dimethyl-5-solanesyl-*p*-benzoquinone (**4b**) was obtained and 0.11 g (65%) pure of [1-^{13}C]-6-bromo-2,3-dimethyl-*p*-benzoquinone (**5b**) was recovered. ^{1}H NMR: δ = 3.41 [dd, ^3J(H-H) = 7.2 Hz and ^3J(C-H) = 4.1 Hz, 2 H, tail 1-CH_2] ppm. ^{13}C NMR: δ = 184.0 (4-^{13}C=O) ppm.

6-Bromo-2,3-dimethyl-5-solanesyl-*p*-benzoquinol: To a separatory funnel was added 0.25 g (0.30 mmol) 6-bromo-2,3-dimethyl-5-solanesyl-*p*-benzoquinone (**4**) in 10 mL ether and 3.0 g $Na_2S_2O_4$ in 30 mL 0.01 M NaOH. This two-layer system was vigorously shaken until the yellow color had disappeared. The water layer was separated and extracted twice with ether. The combined organic layers were washed with sat. NH_4Cl and dried over $MgSO_4$. After concentration under vacuum 0.26 g of crude product was obtained. This crude product was used in the next step without further purification. ^{1}H NMR: δ = 1.60 (s, 27 H, tail 8 CH_3), 1.68 (s, 3 H, tail 36-CH_3), 1.82 (s, 3 H, tail 3′-CH_3), 2.01 (m, 32 H, tail 16 CH_2), 2.13 (s, 3 H, 3′-CH_3), 2.22 (s, 3 H, 2′-CH_3), 3.56 (d, ^3J(H-H) = 6.5 Hz, 2 H, tail 1-CH_2], 4.90 (s, 1 H, 4-OH), 5.11 (m, 9 H, tail 9 CH), 5.30 (s, 1 H, 1-OH) ppm.

[1-^{13}C]-6-Bromo-2,3-dimethyl-5-solanesyl-*p*-benzoquinol: The above described procedure was performed with 0.16 g (0.19 mmol) [1-^{13}C]-6-bromo-2,3-dimethyl-5-solanesyl-*p*-benzoquinone (**4a**) in 5 mL ether and 3.0 g $Na_2S_2O_4$ in 30 mL 0.01 M NaOH to yield 0.16 g of crude product. ^{1}H NMR: δ = 2.22 [d, ^3J(C-H) = 3.8 Hz, 3 H, 2′-CH_3], 5.30 [d, ^2J(C-H) = 1.0 Hz, 1 H, 1-OH] ppm. ^{13}C NMR: δ = 144.2 (1-^{13}COH) ppm.

[4-^{13}C]-6-Bromo-2,3-dimethyl-5-solanesyl-*p*-benzoquinol: The above described procedure was performed with 0.20 g (0.24 mmol) [4-^{13}C]-6-bromo-2,3-dimethyl-5-solanesyl-*p*-benzoquinone (**4b**) in 5 mL ether and 3.0 g $Na_2S_2O_4$ in 30 mL 0.01 M NaOH to yield 0.21 g of crude product. ^{1}H NMR: δ = 2.13 [d, ^3J(C-H) = 4.1 Hz, 3 H, 3′-CH_3], 4.90 [d, ^2J(C-H) = 3.1 Hz, 1 H, 4-OH] ppm. ^{13}C NMR: δ = 146.4 (4-^{13}COH) ppm.

1,4-Di-*O*-bis(methoxymethyl)-6-bromo-1,4-dihydroxy-2,3-dimethyl-5-solanesylbenzene (3)

1,4-Di-*O*-bis(methoxymethyl)-6-bromo-1,4-dihydroxy-2,3-dimethyl-5-[(*all-E*)-3,7,11,15,19,23,27,31,35-nonamethyl-2,6,10,14,18,22,26,30,34-hexatriacontanonaenyl]benzene: A portion of 0.08 g (2.00 mmol) of NaH (60% suspension in mineral oil) was washed three times with PE after which 10 mL very dry THF (distilled from a mixture of THF, Na and benzophenone) was added. To this dispersion was slowly added 0.26 g (0.31 mmol) of 6-bromo-2,3-dimethyl-5-solanesyl-*p*-benzoquinol in 10 mL of very dry THF during which the mixture became dark green. This mixture was then cooled to −15 °C before 0.20 mL (2.64 mmol) chloromethyl methyl ether (Chloromethyl methyl ether is a volatile and cancer suspect agent) in 10 mL very dry THF was added. The mixture was subsequently stirred for 3.5 hours, during which the green color had disappeared, and was poured out into 50 mL saturated NH_4Cl. The organic layer was separated and the water layer was extracted twice with ether. The combined organic layers were washed with saturated NaCl, dried over $MgSO_4$ and the solvents evaporated under vacuum to obtain 0.30 g of crude product. ^{1}H NMR: δ = 1.60 (s, 27 H, tail 8 CH_3), 1.68 (s, 3 H, tail 36-CH_3), 1.77 (s, 3 H, tail 3′-CH_3), 2.01 (m, 32 H, tail 16 CH_2), 2.19 (s, 3 H, 3′-CH_3), 2.25 (s, 3 H, 2′-CH_3), 3.54 [d, ^3J(H-H) = 5.8 Hz, 2 H, tail 1-CH_2], 3.59 (s, 3 H, 4′-OCH_3), 3.66 (s, 3 H, 1′-OCH_3), 4.87 (s, 2 H, 4′-OCH_2O), 5.01 (s, 2 H, 1′-OCH_2O), 5.11 (m, 9 H, tail 9 CH) ppm.

[1-^{13}C]-1,4-Di-*O*-bis(methoxymethyl)-6-bromo-1,4-dihydroxy-2,3-dimethyl-5-solanesylbenzene (3a): The above described procedure was performed with 0.05 g (1.60 mmol) of NaH in 10 mL THF, 0.16 g (0.19 mmol) [1-^{13}C]-6-bromo-2,3-dimethyl-5-solanesyl-*p*-benzoquinol in 10 mL THF and 0.12 mL (1.58 mmol) chloromethyl methyl ether to yield 0.21 g of crude product. ^{1}H NMR: δ = 2.25 [d, ^3J(C-H) = 4.1 Hz, 3 H, 2′-CH_3], 5.01 [d, ^3J(C-H) = 3.4 Hz, 2 H, 1-OCH_2O] ppm. ^{13}C NMR: δ = 149.5 (1-^{13}C) ppm.

[4-^{13}C]-1,4-Di-*O*-bis(methoxymethyl)-6-bromo-1,4-dihydroxy-2,3-dimethyl-5-solanesylbenzene (3b): The above described procedure was performed with 0.06 g (1.92 mmol) of NaH in 10 mL THF, 0.21 g (0.25 mmol) [4-^{13}C]-6-bromo-2,3-dimethyl-5-solanesyl-*p*-benzoquinol in 10 mL THF and 0.16 mL (2.11 mmol) chloromethyl methyl ether to yield 0.22 g of crude product. ^{1}H NMR: δ = 2.19 [d, ^3J(C-H) = 4.1 Hz, 3 H, 3′-CH_3], 4.87 [d, ^2J(C-H) = 3.8 Hz, 1 H, 4-OCH_2O] ppm. ^{13}C NMR: δ = 151.0 (4-^{13}C) ppm.

1,4-Di-*O*-bis(methoxymethyl)-1,4-dihydroxy-2,3-dimethyl-5-solanesylbenzene (2)

1,4-Di-*O*-bis(methoxymethyl)-1,4-dihydroxy-2,3-dimethyl-5-[(*all-E*)-3,7,11,15,19,23,27,31,35-nonamethyl-2,6,10,14,18,22,26,30,34-hexatriacontanonaenyl]benzene: A solution of 0.30 g (0.33 mmol) 1,4-di-*O*-bis(methoxymethyl)-6-bromo-1,4-dihydroxy-2,3-dimethyl-5-solanesylbenzene (**3**) in 15 mL ether was cooled to −70 °C before 1.4 mL (2.24 mmol) butyllithium (1.6 M in hexane) was added. The mixture was stirred for 10 minutes before it was poured into saturated NH_4Cl. The water layer was separated and was extracted twice with ether. The combined organic layers were washed with saturated NaCl solution and dried over $MgSO_4$. After evaporation of the solvent under vacuum 0.29 g of crude product was obtained. ^{1}H NMR: δ = 1.60 (s, 27 H, tail 8 CH_3), 1.68 (s, 3 H, tail 36-CH_3), 1.71 (s, 3 H, tail 3′-CH_3), 2.01 (m, 32 H, tail 16 CH_2), 2.14 (s, 3 H, 3′-CH_3), 2.21 (s, 3 H, 2′-CH_3), 3.55 [d, ^3J(H-H) = 6.9 Hz, 2 H, tail 1-CH_2], 3.48 (s, 3 H, OCH_3), 3.60 (s, 3 H, OCH_3), 4.88 (s, 2 H, 4′-OCH_2O), 5.11 (s, 2 H, 1′-OCH_2O), 5.11 (m, 9 H, tail 9 CH), 6.75 (s, 1 H, 6-CH) ppm.

[1-^{13}C]-1,4-Di-*O*-bis(methoxymethyl)-1,4-dihydroxy-2,3-dimethyl-5-solanesylbenzene (2a): The above described procedure was performed with 0.21 g (0.23 mmol) [1-^{13}C]-1,4-di-*O*-bis(methoxymethyl)-6-bromo-1,4-dihydroxy-2,3-dimethyl-5-solanesylbenzene (**3a**) in 10 mL ether and 1.0 mL (1.6 mmol) butyllithium to yield 0.21 g of crude product. ^{1}H NMR: δ = 2.21 [d, ^3J(C-H) = 3.8 Hz, 3 H,

2′-CH_3), 5.11 [d, ^3J(C-H) = 4.8 Hz, 2 H, 1-OCH_2O], 6.75 [d, ^2J(C-H) = 3.4 Hz, 1 H, 6-CH] ppm. ^{13}C NMR: δ = 151.7 (1-^{13}C) ppm.

[4-^{13}C]-1,4-Di-*O*-bis(methoxymethyl)-1,4-dihydroxy-2,3-dimethyl-5-solanesylbenzene (2b): The above described procedure was performed with 0.22 g (0.24 mmol) [4-^{13}C]-1,4-di-*O*-bis(methoxymethyl)-6-bromo-1,4-dihydroxy-2,3-dimethyl-5-solanesylbenzene (**3b**) in 10 mL ether and 1.0 mL (1.6 mmol) butyllithium to yield 0.21 g of crude product. ^{1}H NMR: δ = 2.14 [d, ^3J(C-H) = 4.1 Hz, 3 H, 3′-CH_3], 4.88 [d, ^2J(C-H) = 3.8 Hz, 1 H, 4-OCH_2O], 6.75 [d, ^3J(C-H) = 9.1 Hz, 1 H, 6-CH] ppm. ^{13}C NMR: δ = 148.6 (4-^{13}C) ppm.

Plastoquinone-9 (1)

2,3-Dimethyl5-[(*all-E*)-3,7,11,15,19,23,27,31,35-nonamethyl-2,6,10,14,18,22,26,30,34-hexatriacontanonaenyl]-*p*-benzoquinol: To a solution of 0.24 g (0.29 mmol) crude 1,4-di-*O*-bis(methoxymethyl)-1,4-dihydroxy-2,3-dimethyl-5-solanesylbenzene (**2**) in 15 mL acetonitrile was added 15 mL H_2O. The mixture was cooled in an ice-bath before 0.50 g (2.99 mmol) 2,6-pyridinedicarboxylic acid and 1.50 g (2.75 mmol) ammonium cerium(VI) nitrate were added. The mixture was stirred for 15 minutes at 0 °C and for 15 minutes at room temperature before 20 mL ether was added. The water layer was separated and extracted twice with ether. The combined organic layers were washed with saturated NH_4Cl, saturated NaCl and dried over $MgSO_4$. After evaporation of the solvent under vacuum, 0.20 g of crude product was obtained. Purification over silica with 15% ether/85% PE as eluens yielded 0.15 g (0.20 mmol, 71%, 80% from **4**) of plastoquinone-9 (**1**). ^{1}H NMR (600 MHz): δ = 1.58 (s, 3 H, tail *cis* 35′-CH_3), 1.60 (s, 21 H, tail 7 CH_3), 1.62 (s, 3 H, tail 3′-CH_3), 1.68 (s, 3 H, tail end *trans* 36-CH_3), 1.98 and 2.06 (m, 32 H, tail 16 CH_2), 2.01 (s, 3 H, 2′-CH_3), 2.03 (s, 3 H, 3′-CH_3), 3.12 [d, ^3J(H-H) = 7.2 Hz, 2 H, tail 1-CH_2], 5.16 [t, ^3J(H-H) = 7.2 Hz, 1 H, tail 2-CH], 5.09 [t, ^3J(H-H) = 6.0 Hz, 1 H, tail 34-CH], 5.11 [br. t, ^3J(H-H) = 6.6 Hz, 7 H, tail 7 CH] 6.47 [t, ^3J(H-H) = 1.7 Hz, 1 H ring CH] ppm. ^{13}C NMR (150 MHz): δ = 12.0 (2′-CH_3), 12.3 (3′-CH_3), 16.0 (m, tail 7′-, 11′-, 15′-, 19′-, 23′-, 27′-, and 31′-CH_3), 16.1 (tail 3′-CH_3), 17.6 (tail end *cis* 35′-CH_3), 25.7 (tail end *trans* 36-CH_3), 26.5 (tail 32-CH_2), 27.4 (tail 1-CH_2), 26.6−26.7 (m, tail 8-, 12-, 16-, 20-, 24-, and 28-CH_2), 39.7 (m, tail 5-, 9-, 13-, 17-, 21-, 25-, 29-, and 33-CH_2), 118.1 (tail 2-CH), 123.8 (tail 6-CH), 124.1−124.4 (m, tail 10-, 14-, 18-, 22-, 26-, 30- and 34-CH), 131.2 (tail 35-C), 132.0 (6-CH), 134.8−135.0 (tail 11-, 15-, 19-, 23-, 27- and 31-C), 135.4 (tail 7-C), 139.6 (tail 3-C), 140.5 (2-C), 140.9 (3-C), 147.9 (5-C), 187.6 (4-C=O), 187.8 (1-C=O) ppm. FT-IR: $\tilde{\nu}$ = 2965 (m), 2943 (m), 2907 (m), 2847 (m), 1644 (s), 1615 (m), 1446 (m), 1384 (m), 1318 (m), 1107 (m), 892 (m), 874 (s), 796 (s), 602 (m), 476 (s), 456 (m), 412 (s) cm^{-1}.

[1-^{13}C]-Plastoquinone-9 (1a): The above described procedure was performed with 0.21 g (0.25 mmol) [1-^{13}C]-1,4-di-*O*-bis(methoxymethyl)-1,4-dihydroxy-2,3-dimethyl-5-solanesylbenzene (**2a**) in 15 mL acetonitrile, 15 mL water, 0.50 g (2.99 mmol) 2,6-pyridinedicarboxylic acid and 1.50 g (2.75 mmol) ammonium cerium(VI) nitrate to yield 0.17 g of crude product. After purification over silica 0.10 g (53%, 69% from **4a**) pure [1-^{13}C]-plastoquinone-9 (**1a**) was obtained. ^{1}H NMR (600 MHz: δ = 2.01 [d, ^3J(C-H) = 3.7 Hz, 3 H, 2′-CH_3] ppm. ^{13}C NMR (150 MHz): δ = 12.0 [d, ^2J(C-C) = 1.5 Hz, 2′-CH_3], 12.3 [d, ^3J(C-C) = 3.8 Hz, 3′-CH_3], 27.4 [d, ^3J(C-C) = 4.4 Hz, tail 1-CH_2], 132.0 [d, ^1J(C-C) = 53.3 Hz, 6-CH], 140.5 [d, ^1J(C-C) = 50.1 Hz, 2-C], 140.9 [d, ^2J(C-C) = 2.0 Hz, 3-C], 147.9 [d, ^2J(C-C) = 1.5 Hz, 5-C], 187.6 [d, ^3J(C-C) = 8.4 Hz, 4-C=O], 187.8 (1-^{13}C=O) ppm. FT-IR: $\tilde{\nu}$ = 2919 (m), 2852 (m), 1646 (s), 1617 (m), 1604 (s), 1448 (s), 1381 (s), 1099 (br. m), 840 (br. m), 418 (m), 412 (m) cm^{-1}.

[4-^{13}C]-Plastoquinone-9 (1b): The above described procedure was performed with 0.21 g (0.25 mmol) [4-^{13}C]-1,4-di-*O*-bis(methoxymethyl)-1,4-dihydroxy-2,3-dimethyl-5-solanesylbenzene (**2b**) in 25 mL acetonitrile, 15 mL water, 0.50 g (2.99 mmol) 2,6-pyridinedicarboxylic acid and 1.50 g (2.75 mmol) ammonium cerium(VI) nitrate to yield 0.21 g of crude product. After purification over silica 0.13 g (69%, 72% from **4b**) pure [4-^{13}C]-plastoquinone-9 (**1b**) was obtained. ^{1}H NMR (600 MHz): δ = 2.03 [d, ^3J(C-H) = 3.7 Hz, 3 H, 3′-CH_3], 6.47 [dt, ^3J(C-H) = 9.9 Hz and ^3J(H-H) = 1.7 Hz, 1 H ring CH] ppm. ^{13}C NMR (150 MHz): δ = 12.0 [d, ^2J(C-C) = 3.7 Hz, 2′-CH_3], 118.1 [d, ^3J(C-C) = 2.3 Hz, tail 2-CH], 132.0 [d, ^1J(C-C) = 1.7 Hz, 6-CH], 139.6 [d, ^4J(C-C) = 1.5 Hz, tail 3-C], 140.5 [d, ^1J(C-C) = 2.0 Hz, 2-C], 140.9 [d, ^2J(C-C) = 51.0 Hz, 3-C], 147.9 [d, ^2J(C-C) = 49.0 Hz, 5-C], 187.6 (4-^{13}C=O), 187.8 [d, ^3J(C-C) = 8.4 Hz, 1-C=O] ppm. FT-IR: $\tilde{\nu}$ = 2918 (vs), 2850 (s), 1661 (m), 1654 (m), 1637 (s), 1608 (vs), 1438 (s), 1381 (s), 1316 (m), 1100 (br. m), 840 (br. m), 418 (m) cm^{-1}.

2,3-Dimethyl-*N*-(phenylsulfonyl)aniline: To a solution of 6.0 mL (49.5 mmol) 2,3-dimethylaniline (**8**) and 4.0 mL (49.5 mmol) pyridine in 160 mL dichloromethane was added 6.4 mL (49.5 mmol) benzenesulfonyl chloride. After stirring overnight the mixture was washed three times with water and dried over $MgSO_4$. After evaporation under vacuum the obtained solid was recrystalized in dichloromethane/PE yielding 12.6 g (97%) of a light pink solid. ^{1}H NMR: δ = 1.94 (s, 3 H, CH_3), 2.20 (s, 3 H, CH_3), 6.64 (br. s, 1 H, NH), 7.01 (br. s, 3 H), 7.3−7.6 (m, 3 H), 7.73 [d, 2 H, ^3J(C-H) = 7.9 Hz] ppm. ^{13}C NMR: δ = 13.7 (CH_3), 20.5 (CH_3), 123.6 (CH), 125.7 (CH), 127.0 (2 CH), 128.3 (CH), 128.8 (2 CH), 132.0, 132.7 (CH), 133.9, 137.7, 139.5 ppm.

***N*-(4,4-Dimethoxy-2,3-dimethylcyclohexa-2,5-dien-1-yl)benzenesulfonamide (26):** A solution of 1.23 g (3.83 mmol) phenyliodonium diacetate in 16.0 mL methanol was injected over 40 min in a solution of 0.50 g (1.91 mmol) 2,3-dimethyl-*N*-(phenylsulfonyl)aniline in 3.7 mL methanol. The solution turned red-yellow and then became yellow within 40 min. Removal of the methanol under vacuum gave a yellow oil which was purified by column chromatography with dichloromethane yielding 0.57 g (93%) product. ^{1}H NMR: δ = 1.90 (s, 3 H, CH_3), 1.94 (s, 3 H, CH_3), 3.17 (s, 6 H, 2 CH_3O), 6.72 [d, 1 H, ^3J(C-H) = 9.4 Hz, 5-H], 7.5−7.7 (m, 3 H), 7.76 [d, 1 H, ^3J(C-H) = 9.4 Hz, 6-H], 8.02 [d, 2 H, ^3J(C-H) = 7.6 Hz] ppm. ^{13}C NMR: δ = 12.3 (3′-CH_3), 13.7 (2′-CH_3), 50.8 (2 CH_3O), 94.7 (4-C), 125.8 (CH), 126.6 (2 CH), 128.6 (2 CH), 132.5 (6-CH), 134.2 (2-C), 141.3 (CS), 143.3 (5-CH), 150.2 (3-C), 163.8 (C=N) ppm.

4,4-Dimethoxy-2,3-dimethyl-*p*-benzoquinone Mono Ketal (27): To a solution of 2.10 g (6.535 mmol) *N*-(4,4-dimethoxy-2,3-dimethylcyclohexa-2,5-dien-1-yl)benzenesulfonamide (**26**) in 110 mL ether and 110 mL 1.0 M KOH solution were added 2.11 g (6.535 mmol) tetrabutylammonium bromide and 1.34 g (7.842 mmol) phenyl bromide. After overnight stirring the phases were separated and the aqueous layer was extracted twice with ether. The combined organic layers were dried over $MgSO_4$. After evaporation under vacuum the crude residue was purified by column chromatography with diethyl ether/PE (40:60) yielding 1.03 g (86%) of a light-yellow oil. ^{1}H NMR: δ = 1.91 (s, 6 H, 2 CH_3), 3.19 (s, 6 H, 2 CH_3O), 6.43 [d, 1 H, ^3J(C-H) = 10.1 Hz, 5-H], 6.73 [d, 1 H, ^3J(C-H) = 10.1 Hz, 6-H] ppm. ^{13}C NMR: δ = 10.8 (3′-CH_3), 13.1 (2′-CH_3), 50.8 (2 CH_3O), 95.7 (4-C), 132.1 (6-CH), 135.2 (2-C), 143.2 (5-CH), 149.2 (3-C), 184.6 (CO) ppm.

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