Synthesis of the Dimethyl Ester of 2′-Cyano-8′-formyl-*N*′-methyl-1′,1a′,5a′,6′-tetrahydroacrido[4,5,5a,6-*bcd*]-Annulated 2,3-Dihydromesoporphyrin – A Novel System with Outstanding Properties as Basis for Photodynamic Therapy in the Far-Red Region of the Visible Spectrum

Richard N S. van der Haas,[a] Siu-Ha Tang,[a] Beatriz Arenas Hernández,[a] Robertus L. P. de Jong,[a] Cornelis Erkelens,[a] Yan Liu,[b] Peter Gast,[b] Threes G. M. Smijs,[c] Hans J. Schuitmaker,[c] and Johan Lugtenburg*[a]

Keywords: Porphyrinoids / Cyclization / NMR spectroscopy / Singlet oxygen / Biological activity

The sterically hindered [5-(2′-cyanovinyl)mesoporphyrin dimethyl ester]nickel complex **1** has been treated with *N*-methylformanilide under Vilsmeier conditions. Besides the expected *meso*-formylporphyrin derivatives, 22% of a novel green compound was isolated. After removal of Ni^{2+} by treatment with concentrated sulfuric acid, the structure of the novel compound was elucidated by mass spectrometry and ^1H and ^{13}C NMR spectroscopy, which led to the unambiguous establishment of the unprecedented racemic dimethyl ester of 2′-cyano-8′-formyl-*N*′-methyl-1,1a,5a,6-tetrahydroacrido[4,5,5a,6-*bcd*]-annulated 2,3-dihydromesoporphyrin structure **5**. This racemic form was efficiently separated into its two pure enantiomeric forms each of which shows the same electronic spectrum as the racemate mixture. The two optical isomers show the exact opposite circular dichroism spectra. The structure of this novel product is clearly formed after the initial attack of the carbenium ion formed from *N*-methylformanilide and $POCl_3$ at the α-carbon atom of the appending *meso*-acrylonitrile function. This process is thus far an unprecedented cyclization process under Vilsmeier conditions. It is clear that in this case the aromatic system of the Vilsmeier reagent is intimately involved in this reaction. In order to further explore the reactivity of the starting material, we treated it with the Vilsmeier reagent prepared from dimethylformamide and $POCl_3$. In this case we obtained, besides the expected formylation products, a mixture of two *peri*-annulated quinoporphyrins. From the structures of the products and the starting material it could be established that in this case the attack of the Vilsmeier reagent is on the nitrogen atom of the appending acrylonitrile function. For the initial tests of **5** as the basis of a photodynamic therapy we found that in the presence of air and light it is an efficient singlet oxygen generator that is stable during the irradiation time. It shows no toxicity towards lung carcinoma cells in the dark while in the presence of air and light the compound leads to a rapid killing of the cancer cells at concentrations that are an order of magnitude lower than in the case of the quinoporphyrin systems. These are all promising properties for continued studies and further development towards a photodynamic cancer therapy.

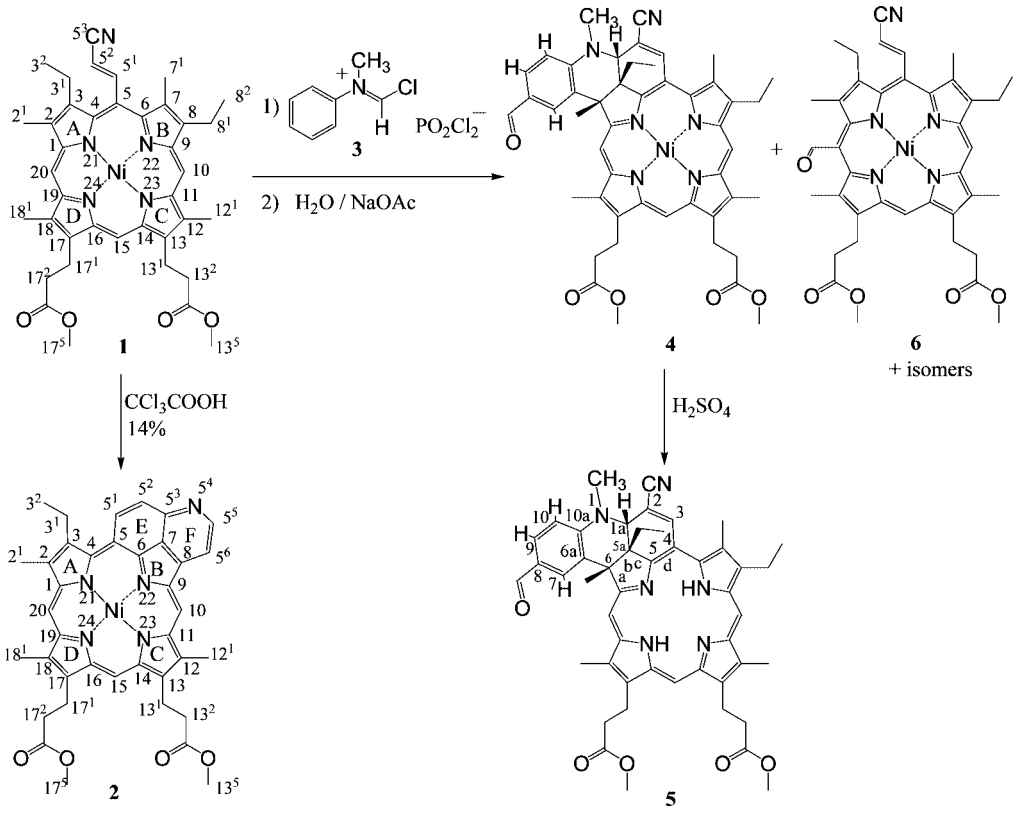
Introduction

Recently we published that the sterically congested [5-(2′-cyanovinyl)mesoporphyrin dimethyl ester]nickel complex **1** when treated with acid at high temperature in a one-pot reaction gave a 14% yield of the corresponding dimethyl ester of quino[4,4a,5,6-*efg*]-annulated (8-deethyl-7-demethylmesoporphyrin)nickel complex **2** (see Scheme 1).[1] This reaction is unique, because in a series of steps two aromatic rings are annulated to the porphyrin system. We also found that this is a general conversion for congested *meso*-acrylonitrile-substituted porphyrins. Compound **2** could simply be demetalated with concentrated sulfuric acid leading to a porphyrin that shows all the positive physical, chemical, and biological properties that are essential for the development of this molecule as a basis for a photodynamic cancer therapy in the far-red region of the visible spectrum.

The one-pot formation of **2** from **1** by a proton-induced reaction may be the first product of a treasure trove of very useful novel systems that may be simply derived from **1**. In order to further explore the reactivity of **1** with electrophilic reagents we started a study to treat **1** with carbenium ions as electrophiles. In this paper we describe the results obtained from the reaction of **1** with (chloromethylene)methyl(phenyl)ammonium dichlorodioxophosphate (**3**). This salt is formed from the reaction between *N*-methylformanilide and phosphorus oxychloride and commonly used in the

[a] Leiden University, Leiden Institute of Chemistry, Gorlaeus Laboratory,
P. O. Box 9502, 2300 RA Leiden, The Netherlands
Fax: +31-71-5274488
E-mail: lugtenbu@chem.leidenuniv.nl
[b] Department of Biophysics, Huygens Laboratory,
P. O. Box 9504, 2300 RA, Leiden, The Netherlands
[c] Leiden University Medical Center, Sylvius Laboratory,
P. O. Box 9503, 2300 RA Leiden, The Netherlands

 DOI: 10.1002/ejoc.200500174



Scheme 1. The reactions of [5-(2′-cyanovinyl)mesoporphyrin dimethyl ester]nickel complex by (chloromethylene)(methyl)(phenyl)ammonium dichlorophosphate (**3**) giving **4** and **6** and by a proton giving **2**. All names are according to semisystematic IUPAC nomenclature: **1**: [5-(2′-cyanovinyl)mesoporhyrin dimethyl ester]nickel complex; **2**: quino[4,4a,5,6-*efg*]-annulated 8-deethyl7--demethylmesoporphyrin dimethyl ester; **3**: (2′-cyano-8′-formyl-*N*′-methyl-1,1a,5a,6-tetrahydroacrido[4,5,5a,6-*bcd*]-annulated 2,3-dihydromesoporphyrin dimethyl ester)nickel complex; **4**: 2′-cyano-8′-formyl-*N*′-methyl-1,1a,5a,6-tetrahydroacrido[4,5,5a,6-*bcd*]-annulated 2,3-dihydromesoporphyrin dimethyl ester; **5**: [20-formyl-5-(2′-cyanovinyl)mesoporphyrin dimethyl ester]nickel complex.

Vilsmeier–Haack formylation of conjugated systems. We hoped that besides the formation of various *meso*-formyl derivatives novel products would also be obtained, resulting from the special reactivity of the sterically congested acrylonitrile structure of **1**.

Results and Discussion

Synthesis

The [5-(2′-Cyanovinyl)mesoporphyrin dimethyl ester]-nickel complex was prepared according to our earlier paper[1] and treated with a sixfold excess of Vilsmeier–Haack reagent prepared from *N*-methylformanilide and phosphorus oxychloride After hydrolysis and purification on silica gel, 130 mg (22%) of a novel green compound **4** with a strong absorption at 650 nm (Figure 1) was obtained along with a mixture of the expected *meso*-formylated products. From the latter mixture the pure [20-formyl-5-(2′-cyanovinyl)mesoporphyrin dimethyl ester]nickel complex **6** could be isolated.

Besides a strong absorption at 647 nm, compound **4** also shows a strong peak at 2198 cm^{-1} in the infrared spectrum indicating the presence of a nitrile function. Peaks are also present at 1676 cm^{-1} and a Fermi doublet at 2730 and 2802 cm^{-1}, indicating that there is an aldehyde group attached to an electron-rich part of the molecule. HR-ESI mass spectrometry gave an exact mass of *m*/*z* = 847.3133, which corresponds with an elemental composition of $^{12}C_{48}{}^{1}H_{49}{}^{14}N_{6}{}^{58}Ni^{16}O_{5}{}^{+}$ (calculated: *m*/*z* = 847.3118),

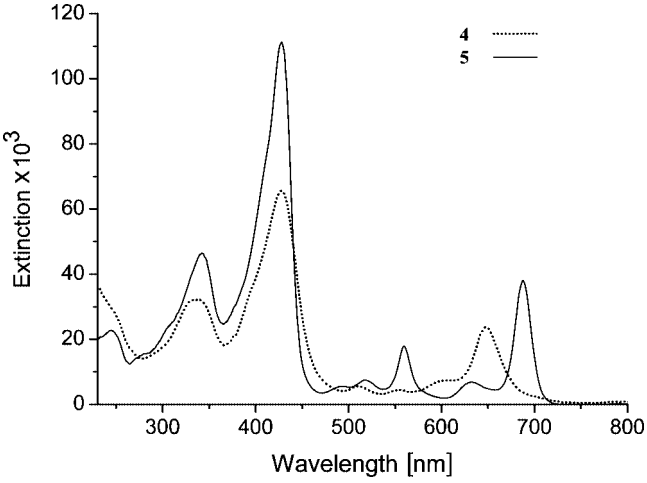


Figure 1. UV/Vis spectra of the nickel complex **4** and the free base **5** of 2′-cyano-8′-formyl-*N*′-methyl-1,1a,5a,6-tetrahydroacrido[4,5,5a,6-*bcd*]-annulated 2,3-dihydromesoporphyrin dimethyl ester.

which is the monoprotonated form of **4**. Removal of nickel with concentrated sulfuric acid gave the nickel-free derivative **5** in 63% yield, of which the long-wavelength absorption has shifted to 687 nm (Figure 1). The nitrile function and the aldehyde function are still present as indicated by the absorptions in the IR spectrum at 2194 cm^{-1}, 1674 cm^{-1} and a Fermi doublet at 2720 and 2760 cm^{-1}. A small amount of the analogous compound, in which the nitrile function was hydrolyzed to the corresponding amide (20%), was also obtained. This amide compound shows no nitrile absorption in the IR spectrum; instead amide I and II peaks are observed at 1667 (overlaps with aldehyde) and 1653 cm^{-1}, respectively. The Fermi resonances at 2730 and 2765 cm^{-1} of the aldehyde are still present.

The HR-ESI mass spectrum of **5** shows the parent peak at *m*/*z* = 791.3931 which corresponds to an elemental composition of $^{12}C_{48}{}^{1}H_{51}{}^{14}N_{6}{}^{16}O_{5}{}^{+}$ (calculated *m*/*z* = 791.3921). This corresponds to the monoprotonated form of **5** with formula $^{12}C_{48}{}^{1}H_{50}{}^{14}N_{6}{}^{16}O_{5}$. Compared to **4**, the Ni^{2+} ion has been substituted by two hydrogen atoms. The unsaturation number is 27. The elemental composition of 5-(2′-cyanovinyl)mesoporphyrin dimethyl ester is $^{12}C_{39}{}^{1}H_{43}{}^{14}N_{5}{}^{16}O_{4}$ with an unsaturation number of 21. This means that **5** contains nine more carbon atoms, seven more hydrogen atoms, one more nitrogen and one more oxygen atom than 5-(2′-cyanovinyl)mesoporphyrin dimethyl ester. Also, the unsaturation number in **5** has increased by six units. These facts indicate that the carbon, hydrogen and nitrogen atoms of reagent **3** have been included in the structure of **5** and that in addition an aldehyde group is present, presumably attached to the benzene ring of the *N*-methylaniline group that is now incorporated into structure **5**.

NMR Spectroscopy

NMR spectroscopy is the method of choice for establishing the connections between the atoms in a molecule. The structure and semi-systematic IUPAC numbering of **5** is given in Figure 2A. This numbering is used for the assignment of the various ^{1}H, ^{13}C, and ^{15}N signals in the NMR study. The 600 MHz ^{1}H NMR spectrum of **5** is reproduced in Figure 3. Comparing the ^{1}H NMR spectrum of **5** with that of the [5-(2′-cyanovinyl)mesoporphyrin dimethyl ester] nickel complex shows that the AB doublet of the cyanovinyl group has disappeared. All other signals of the starting material are still present. In this case they are considerably shifted, indicated with the letter S in Figure 3. The signals indicated by A are absent in the spectrum of the starting material. The peaks at δ = 7.60 (^{4}J = 1.5 Hz), 7.21 (^{3}J = 8.9, ^{4}J = 1.5 Hz) and 6.49 (^{3}J = 8.9 Hz) ppm form an aromatic spin system due to a 1,2,4-trisubstituted benzene nucleus. The aldehyde peak at δ = 9.14 ppm shows a nuclear Overhauser effect (NOE) with the peaks at δ = 7.60 and 7.21 ppm (Figure 2A). The peak at δ = 3.56 ppm must be due to the NCH_3 group, because it shows an NOE with the signal at δ = 6.49 ppm. The signal at δ = 4.82 ppm shows a small coupling of 0.6 Hz with the signal at δ = 9.40 ppm. The methyl signal at δ = 2.64 ppm must be due to a methyl group attached to an sp^3-carbon atom. The same applies to the signals at δ = 1.91 and 1.67 ppm, which are methylene signals of an ethyl group at a chiral sp^3 center.

In Figure 2A all the nuclear Overhauser interactions are given; based on the discussion above, the signals of all hydrogen atoms bound to the carbon skeleton can now be unequivocally assigned. This supports the structure for **5** as has been depicted in Scheme 1 and in Figure 2.

Based on the ^{1}H NMR spectrum, the presence of an unprecedented *peri*-condensed (*N*-methyl-1,1a,5a,6-tetrahydroacrido)porphyrin system has been established.

In order to establish this structure rigorously, we used ^{13}C NMR spectroscopy. In Figure 4 the attached proton test ^{13}C NMR spectrum between 200 and 90 ppm is reproduced. The signals that point downwards are those of car-

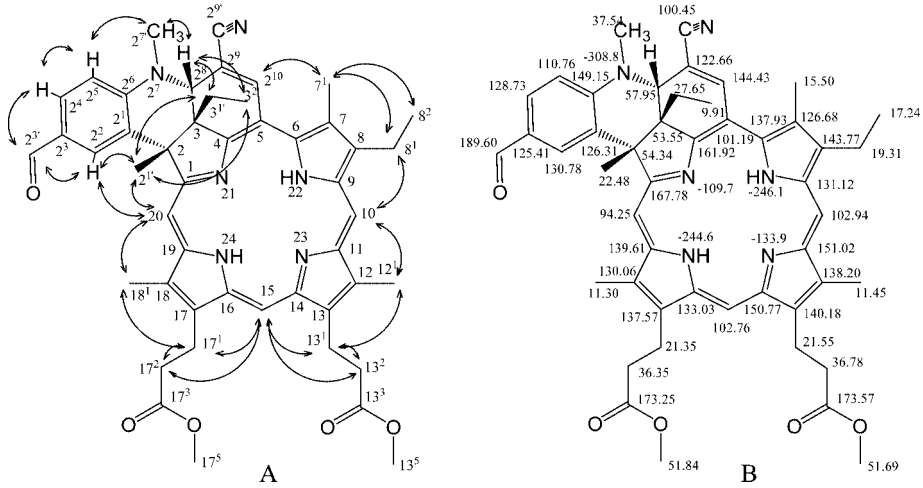


Figure 2. A: Structure and numbering of 2′-cyano-8′-formyl-*N*′-methyl-1,1a,5a,6-tetrahydroacrido[4,5,5a,6-*bcd*]-annulated 2,3-dihydromesoporphyrin dimethyl ester **5** according to the semisystematic IUPAC rules for tetrapyrroles. This numbering is used for the NMR spectroscopic studies. The NOE interactions in the ^{1}H NMR spectra are indicated by doubly pointed arrows. B: The ^{13}C chemical shift values of all carbon atoms and the ^{15}N chemical shift values of the aniline nitrogen atom and all central porphyrin nitrogen atoms are indicated.

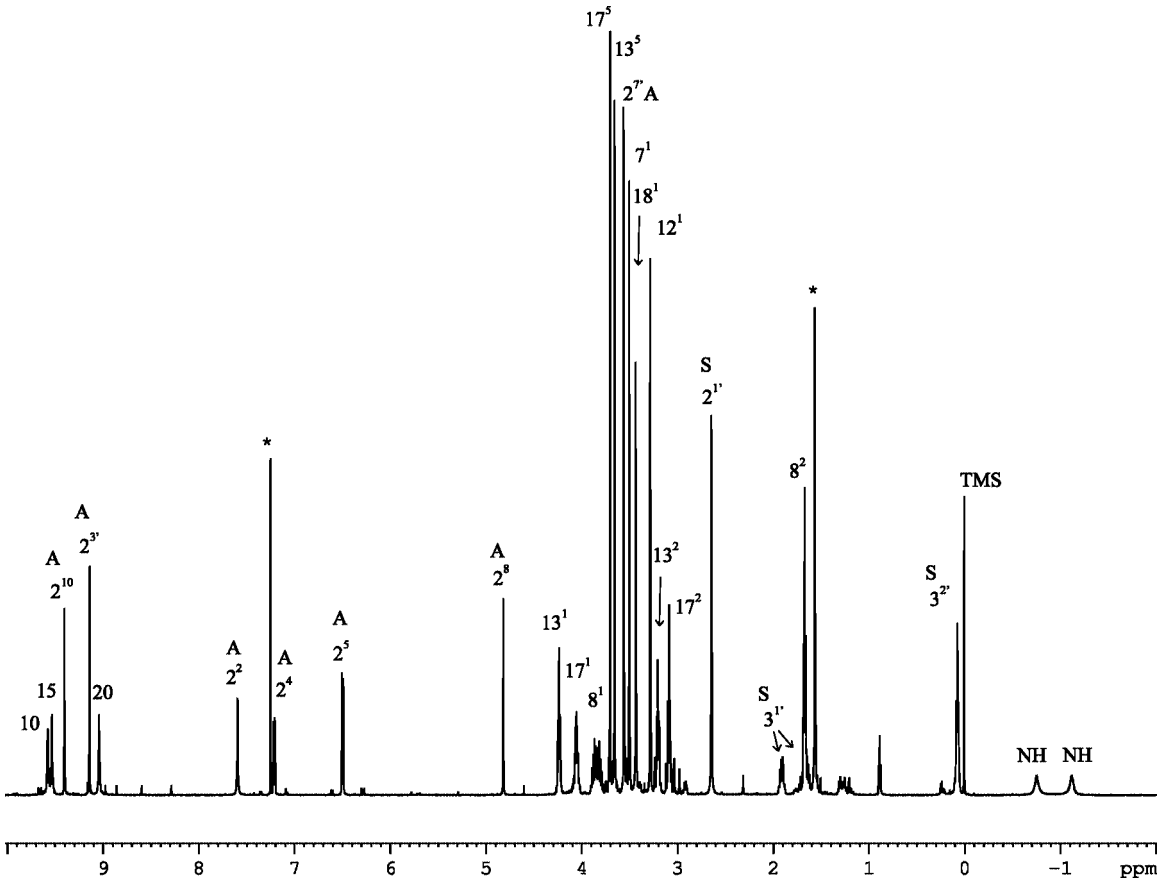


Figure 3. 600 MHz ^{1}H NMR spectrum of **5** dissolved in $CDCl_3$ with tetramethylsilane as the internal standard. The peaks marked with an * are from $CHCl_3$ and H_2O. The peaks marked with A are absent in the starting compound [5-(2′-cyanovinyl)mesoporphyrin dimethyl ester]nickel complex **1**. The resonances marked with S are considerably shifted compared to those of the starting compound **1**. Besides these peaks, signals of a minor impurity are observable throughout the whole spectral range.

bon atoms directly attached to hydrogen atoms. The signals of the quaternary carbon atoms point upwards. The tertiary carbon signals could be assigned with the use of 2D ^{1}H–^{13}C heteronuclear spin quantum coupling (HSQC) NMR spectroscopy (see Figure 4). With 2D ^{1}H–^{13}C heteronuclear multiple bond correlation (HMBC) NMR spectroscopy especially sp^2 $^3J(^{13}C–^1H)$ interactions of 8 Hz are revealed, also some ^2J and $^4J(^{13}C–^1H)$ interactions are observed. In Figure 2A it can be seen that the aromatic carbon atom at position 2^1 only has unique $^3J(^{13}C–^1H)$ interactions with the protons at positions $2^{1\prime}$ and 2^5. The only carbon signal that shows these interactions in the 2D ^{1}H–^{13}C HMBC spectrum is that at δ = 126.3 ppm, which is therefore ascribed to carbon atom 2^1. The carbon resonance at δ = 149.2 ppm shows $^3J(^{13}C–^1H)$ interactions with the protons at positions 2^2, 2^4, $2^{7\prime}$ and 2^8. Only the carbon atom 2^6 is in the right position to show these four interactions. The carbon signal of the nitrile group is found at δ = 100.5 ppm and shows $^3J(^{13}C–^1H)$ interactions with the protons at positions 2^8 and 2^{10}. In a similar way all the quaternary carbon atoms of compound **5** show unique long-range ^{1}H–^{13}C interactions making it possible to assign all the carbon signals as indicated in Figure 2B.

For the 2D ^{1}H–^{15}N HMBC spectrum similar rules apply for the ^{15}N NMR assignments as discussed above for the ^{13}C case. However, the natural abundance of ^{15}N is only 0.35% instead of the 1.1% for ^{13}C. The ^{15}N signals could be observed indirectly from the very sensitive proton signals using the above-mentioned technique. These spectra show the signals of five different nitrogen atoms at δ = –109.7, –133.9, –244.6, –246.1 and –308.8 ppm with the ^{15}N signal of nitromethane as a reference. The assignments of these nitrogen atoms are given in Figure 3. The nitrogen atom at position 23 has a $^3J(^{15}N–^1H)$ interaction with the protons at positions 10 and 15. The nitrogen signal at δ = –133.9 ppm shows these connectivities and is therefore assigned to N23. The chemical shift values of δ = –109.7 and –133.9 ppm correspond to those of sp^2-nitrogen atoms, and those of δ = –246.1 and –244.6 ppm are in the expected region for pyrrole nitrogen atoms. The signal at δ = –308.8 ppm shows connectivities with the proton at position 2^5 and a $^2J(^{15}N–^1H)$ interaction with the methyl protons at position $2^{7\prime}$. It is therefore assigned to the nitrogen atom in the tetrahydroacrido system condensed to the porphyrin. The chemical shift value of δ = –308.8 ppm is in the region of the aliphatic ^{15}N values in agreement with the

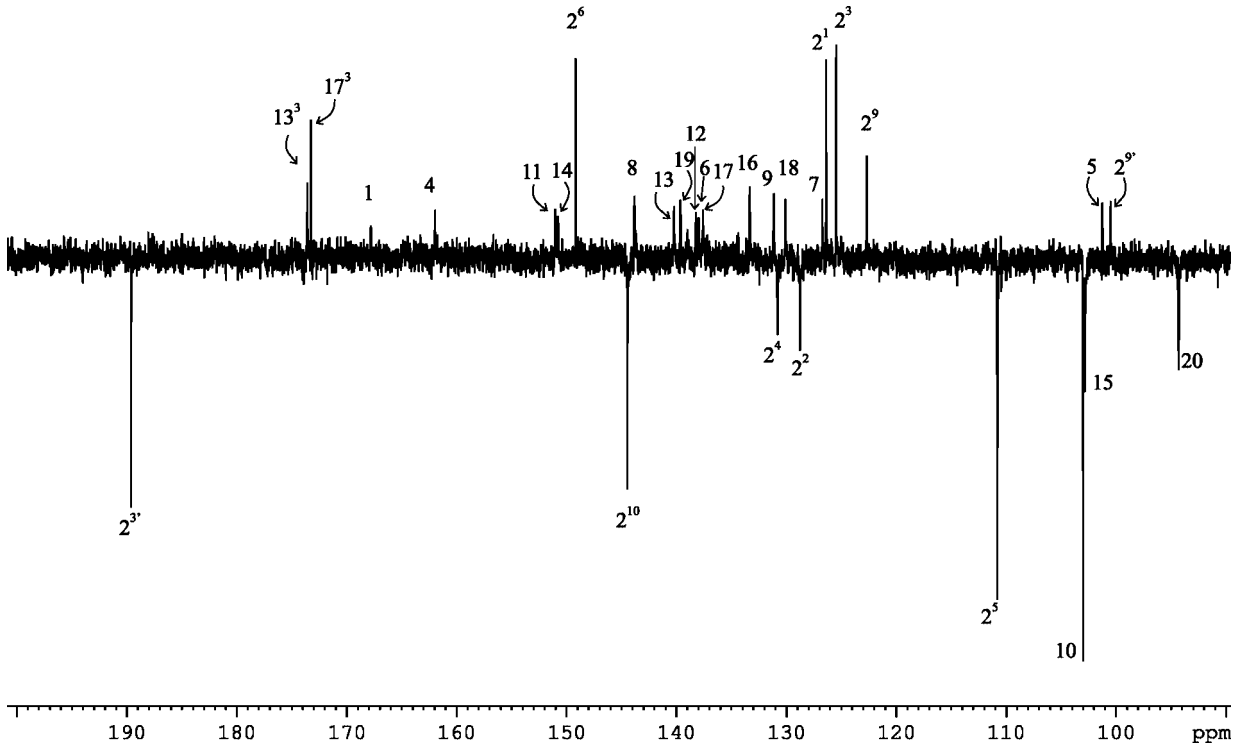


Figure 4. Assignment of all sp^2-carbon signals in the 150 MHz ^{13}C-attached proton test NMR spectrum of **5**.

structure. The nitrile nitrogen atom could not be detected, which is in agreement with the structure since this nitrogen atom is located four bonds away from the nearest hydrogen atom, too far for detection by the ^1H–^{15}N HMBC interaction. Also, the NMR spectroscopy did not allow the unambiguous assignment of the NH protons of the porphyrin structure at δ = –0.76 and –1.12 ppm. It is clear that NMR spectroscopy has led to the unambiguous establishment of the complete assignment of all ^{13}C signals, almost all ^1H signals except those for H22 and H24 and for 5 of the 6 ^{15}N signals.

The structure of **5** has three chiral carbon atoms (2, 3 and 2^8). However, due to the bridging aniline structure at positions 2 and 2^8, the substituents on the carbon atoms 2, 3, and 2^8 are forced into a *syn*,*syn* orientation. This results in the fact that **5** occurs in only two enantiomeric forms. These two enantiomers have been fully separated with HPLC using a Chiralcel OD column and a hexane/ethanol mixture (75:25, v/v) as the eluent. The chromatogram is reproduced in Figure 5. The CD spectrum of enantiomer 1 is reproduced in Figure 6. The CD spectrum of the other enantiomer is the exact mirror image of that of enantiomer

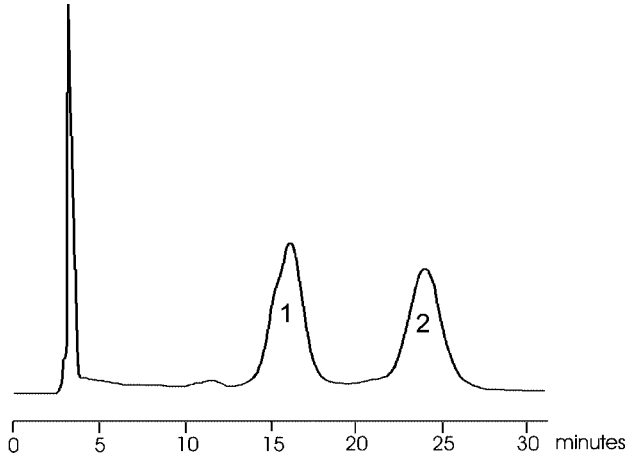


Figure 5. Separation of the two enantiomers of 2′-cyano-8′-formyl-*N*′-methyl-1,1a,5a,6-tetrahydroacrido[4,5,5a,6-*bcd*]-annulated 2,3-dihydromesoporphyrin dimethyl ester **5** using HPLC with a Chiralcel OD column and a mixture of hexanes and ethanol (75:25, v/v) as the eluent.

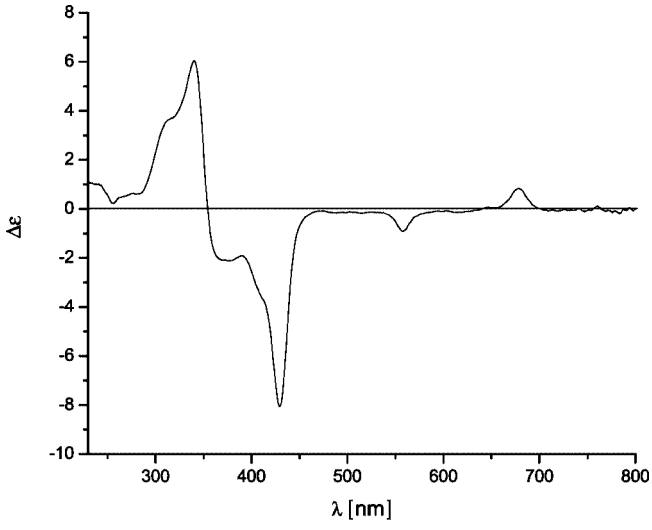


Figure 6. CD spectrum of the faster running enantiomer of **5** in CH_2Cl_2.

1. Both enantiomers have electronic absorption spectra identical to that reproduced for **5** depicted in Figure 1. These results show that **5** is indeed a 1:1 mixture of two enantiomers, which are easily separated into the optically pure forms on a semi-preparative scale.

Reaction Mechanism

Comparison of the structure of the starting material **1** with that of the product shows that all the carbon and nitrogen atoms of reagent **3** have been incorporated into **4**. Also an additional aldehyde group has been incorporated *para* to the *N*-methylaniline structure. Scheme 2 is based on the facts discussed above and indicates a reaction mechanism for the formation of **4**.

Electrophilic attack of the carbenium ion (chloromethylene)(methyl)(phenyl)ammonium dichlorophosphate (**3**) on carbon atom 5^2 of the acrylonitrile function in **1** gives, after the loss of a proton from position 5^2 and a Cl^- ion loss from the Vilsmeier reagent, the stabilized allylic cation depicted in **A**. Intramolecular attack of this allylic cation on pyrrolic carbon atom 3 in ring A gives intermediate **B** in which a new six-membered ring is formed such that the ethyl group in position 3 and the appending *N*-methylaniline group are oriented *trans* to one another and a new carbenium ion center is generated on atom 2. This carbenium atom at C2 in intermediate **B** attacks the *ortho*-carbon atom of the *N*-phenyl group, which after proton loss and reformation of the aromatic ring gives intermediate **C**. In intermediate **C** the very electron-rich *para*-C8 is present and reacts with the Vilsmeier reagent **3**, which is present in excess and after workup leads to an efficient introduction of a formyl group on the aniline substructure in **4**.

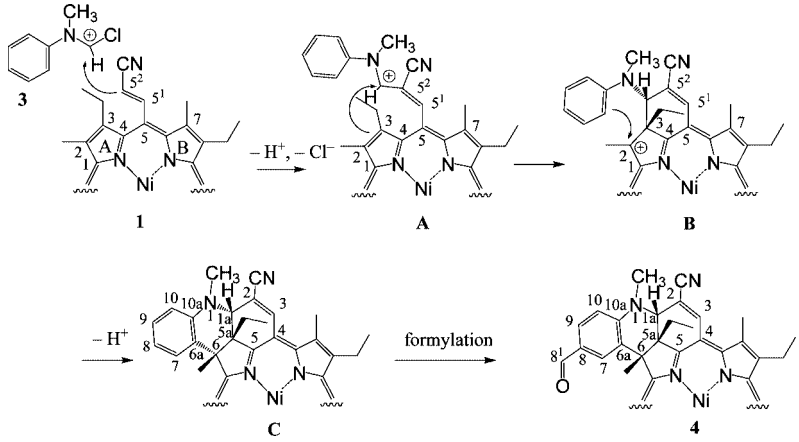
Although many cyclizations under Vilsmeier conditions have been described[2] the cyclization that results in the formation of **4** during Vilsmeier formylation of **1** has never been encountered before. It is clear that the steric hindrance of the acrylonitrile function in **1** leads to this unique cyclic product formation.

It is to be expected that this reaction has a very wide scope because Vilsmeier reagents with several substituted acylanilines and other aromatic acylamides such as α- and β-naphthyl, 9-phenanthryl, anthracyl and pyryl, etc. are easily available.

This means that a whole series of the analogs of **5** are in principle easily accessible. Also the aldehyde function in **5** can easily be converted into various different organic functions. This situation will make the discovery of a system that fulfils the requirements for the basis of a photodynamic therapy attainable in an optimal way.

Scheme 2 indicates that after attack of the Vilsmeier reagent on carbon atom 5^2 of the 5-acrylonitrile function in **1**, the second attack is on C3 of ring A in the porphyrin system. In principle a similar product may be formed when the second attack takes place at C7 of ring B in **1**. In the ^{1}H NMR spectrum of nickel complex **4** very weak signals of a minor impurity can be observed showing a similar pattern as that of **4**. Although these signals are too weak to give certainty about the identity of this impurity they can probably be assigned to the product formed when the second attack takes place at carbon atom 7 of ring B in **1**. Unfortunately, due to its low abundance, this impurity could not be isolated. It is interesting that this latter type of product is formed in such a very low yield. Probably because of subtle factors that are involved in the selectivity between reaction paths that lead to the observed product in favor of the other possible reaction course. Perhaps the ethyl group is slightly more out of plane than the methyl group making the approach of the Vilsmeier reagent at ring A of the porphyrin moiety easier. Also the orientation of the acrylonitrile function may play a role.

From the structure of **4** and the reaction mechanism of its formation it is clear that the phenyl ring in the Vilsmeier reagent derived from *N*-methylformamide has an essential role in the formation of product **4**. When using the Vilsmeier reagent derived from dimethylformamide, the [20-formyl-5-(2^1-cyanovinyl)mesoporphyrin dimethyl ester]nickel complex **6** and its isomers could be isolated. Besides these



Scheme 2. Mechanism for the formation of the *peri*-condensed tetrahydroacrido-dihydromesoporphyrin **4** starting from the nickel complex of 5-(2′-cyanovinyl)mesoporphyrin dimethyl ester **1**. For the numbering of the atoms in **A** and **B** the semisystematic IUPAC numbering of **1** is used. For **C** and **4** that of Scheme 1 is used.

expected products, a mixture of two novel products **7** and **8** could also be isolated. This mixture shows a strong absorption in the visible spectrum at 633 nm. The HR-FAB mass spectrum shows two parent peaks, one large signal at m/z = 684.2134 corresponding with an elemental composition $^{12}C_{38}{}^{1}H_{36}{}^{14}N_5{}^{16}O_4{}^{58}Ni^+$ and a smaller signal at m/z = 698.2283 with composition $^{12}C_{39}{}^{1}H_{38}{}^{14}N_5{}^{16}O_4{}^{58}Ni^+$. From the ^{1}H NMR spectrum it can be observed that the main compound shows two AB spectra at δ = 7.90 and 8.91 ppm with ^3J = 8.9 Hz, and at δ = 8.17 and 9.31 ppm with ^3J = 4.6 Hz. Using 2D ^{1}H NOESY NMR spectroscopy and comparing this with the analytical properties of **2**[1] we found that the structure of the main compound is **7** as depicted in Figure 7. The minor compound has one carbon and two hydrogen atoms more than **7**. In the ^{1}H NMR spectrum an AB pattern corresponding to the benzene part of the quinoline ring system at δ = 8.08 and 9.05 ppm with ^3J = 9.0 Hz is observed. The ^{1}H NMR spectrum of this compound differs from that of **7** in that the AB pattern due to the pyridine part of the quinoline is not observed; instead it shows one extra methyl signal at δ = 2.87 ppm as a singlet and a singlet at δ = 9.03 ppm. From 2D ^{1}H NOESY NMR spectroscopy and comparison with the analytical properties of **2** this compound turns out to be the 5^6-methyl-substituted quinoporphyrin **8** as depicted in Figure 7. Based on our earlier detailed structure elucidation of *peri*-annulated quinoporphyrin systems[1] and the 600 MHz ^{1}H NMR spectrum of the mixture of **7** and **8**, the assignment of all signals could be performed and the structures of **7** and **8** established without any ambiguity.

It is interesting to realize that the reaction of **1** with the Vilsmeier reagent obtained from *N*,*N*-dimethylformamide gives completely different products from that of the Vilsmeier reagent **3** obtained from *N*-methylformanilide. In the latter case only attack at C2 of the acrylonitrile function and subsequent cyclization of the aniline unit is observed. In the case of the reagent obtained from dimethylformamide and phosphorus oxychloride only attack at the nitrogen atom of the acrylonitrile is observed. Products from attack at C2 of the acrylonitrile function have not been detected. In the latter case the cyclization involves the chloromethylene carbon atom of the Vilsmeier reagent into the quinoline ring (Scheme 3) and a more rational way of preparing *peri*-annulated quinoporphyrins is realized. Once again this type of cyclization has never been observed in Vilsmeier reactions.[2] Presumably the much less delocalized positive charge in the Vilsmeier reagent from dimethylformamide and its more hard-electrophilic character switch the reaction type over to the reaction type we have described before in the reaction of **1** with the hard proton.[1] It is interesting to realize that in the case of a Vilsmeier reaction with the reagent from dimethylformamide both expected cyclization products are formed. In the present case compound **7**, which is the main product, is formed from the attack on C3 of the pyrrole ring A while the minor product **8** is formed from the attack on C7 of pyrrole ring B. This means that the same electronic and steric factors are present during this Vilsmeier cyclization reaction of **1** as in the case with *N*-methylformanilide where compound **4** results from the electrophilic attack on C3 in pyrrole ring A as the sole observed product.

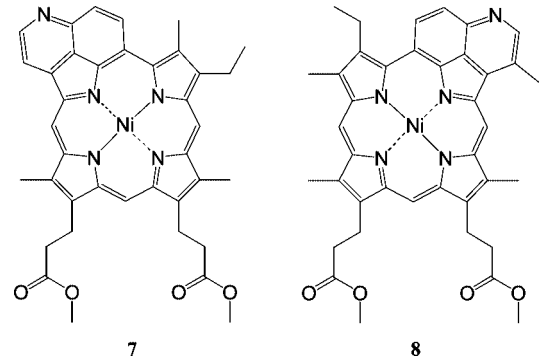
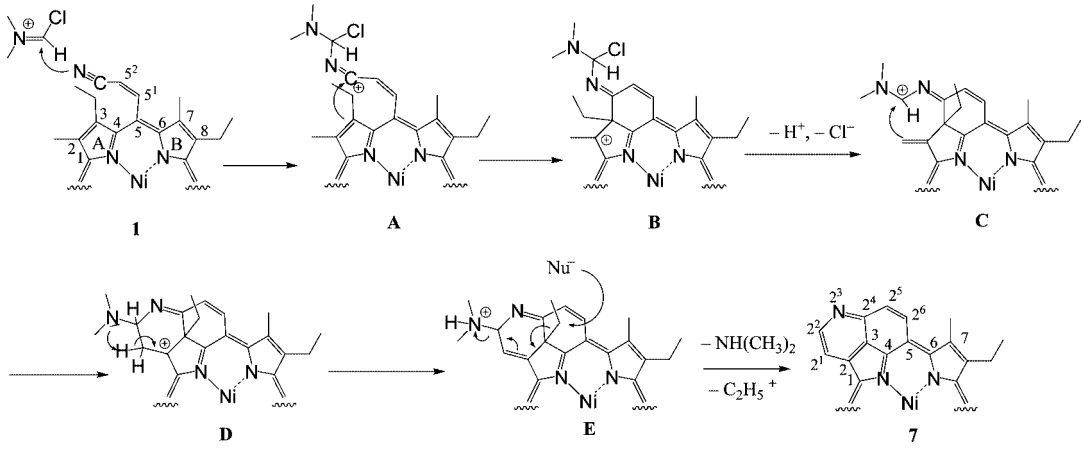


Figure 7. Structures of (3-deethyl-2-demethylquino[4,4a,5,6-*bcd*]-mesoporphyrin dimethyl ester)nickel complex **7** and (8-deethyl-7-demethyl-3′-methylquino[4,4a,5,6-*efg*]mesoporphyrin dimethyl ester)nickel complex **8** formed by attack of (chloromethylene)dimethylammonium dichlorophosphate on the nitrogen atom of the nitrile function in **1**.



Scheme 3. Mechanism for the formation (quino[4,4a,5,6-*bcd*]-annulated 3-deethyl-2-demethylmesoporphyrin)nickel complex **7** from the nickel complex of 5-(2′-cyanovinyl)mesoporphyrin dimethyl ester **1**.

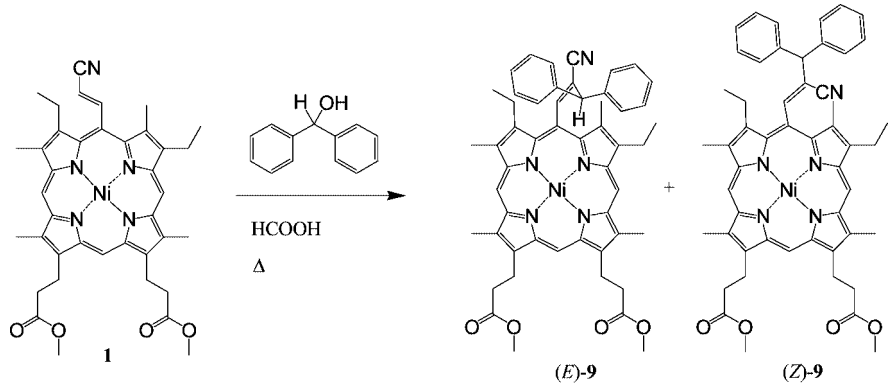
Scheme 3 rationalises how **7** and **8** can be formed. First, an attack of the (chloromethylene)dimethylammonium cation on the nitrile nitrogen atom takes place to form a carbenium ion that attacks C3 of the pyrrole ring A forming intermediate **B**, which will lead to compound **7**. This intermediate looses a proton to form the exomethylene structure in **C** and subsequently chlorine to form an amidinium structure. Intramolecular attack involving intermediate **D** and subsequent proton rearrangement gives the protonated dimethylamine derivative of a dihydropyridine structure which, after removal of dimethylamine and nucleophilic attack on the quaternary ethyl group leading to its elimination, gives the stable quinoporphyrin **7**. A similar reaction mechanism can be written for the formation of **8**; the only difference is that the carbenium ion in intermediate **A** attacks C7 of pyrrole ring B. Interestingly, a completely novel preparation for quino-annulated porphyrins has been realized without the occurrence of an oxidation step. Products that result from the possible attack of the (chloromethylene)dimethylammonium cation on position 5^2 of the unsaturated acrylonitrile function could not be detected.

Recently, it has been reported that the diphenylcarbenium ion (formed by reaction of diphenylmethanol in formic acid) attacks the nitrogen atom of benzonitrile;[3] the diphenylcarbenium ion is a softer cation than the (chloromethylene)dimethylammonium cation because its positive charge is much more delocalized. It is therefore interesting to see if its reaction with the acrylonitrile group in **1b** would take place on the nitrile nitrogen atom as with the (chloromethylene)dimethylammonium cation or whether it would resemble the reactivity of Vilsmeier reagent **3** by attacking the carbon atom 5^2 of the acrylonitrile.

Upon treatment of **1** with 1 equiv. of diphenylmethanol in hot formic acid, two compounds were isolated and characterized as the 5^2-benzhydryl-substituted products (*Z*)-**9**, (*E*)-**9** (Scheme 4). Based on ^{1}H NMR spectroscopy it is established that (*Z*)-**9** and (*E*)-**9** are geometrical isomers that result from the attack of the diphenylcarbenium cation on C5^2 of the acrylonitrile function in **1**. Whereas the ^{1}H NMR spectrum of (*Z*)-**9** does not show special properties, that of (*E*)-**9** shows upfield shifts of the diphenylmethyl hydrogen signal and the hydrogen signals of the diastereotopic phenyl groups, which are now magnetically nonequivalent. The chemical shift of the diphenylmethyl hydrogen atom in (*E*)-**9** is 2.68 ppm upfield compared to that of (*Z*)-**9**. This indicates that the diphenylmethyl hydrogen atom is close to the porphyrin macrocycle. The *ortho*-hydrogen atoms of the two diastereotopic phenyl rings have their signals at δ = 6.11 and 6.17 ppm. The signal at δ = 6.11 ppm shows an NOE with the neighboring methyl group of pyrrole B while the signal at δ = 6.17 ppm shows an NOE with the neighboring ethyl group of pyrrole ring A.

Photochemical Singlet Oxygen Generation

Compound **5** dissolved in toluene was excited with a 9 mJ, 15 ns laser pulse at 532 nm from a home-built Q-switched frequency doubled Nd:YAG laser operating at 10 Hz. Emission from singlet oxygen at 1270 nm was detected at 90° to the quartz cuvette by an InGaAs photodiode, amplified and then signal-averaged (256 shots) on a digital oscilloscope (HP infinium). A 1260 nm interference filter (FWHM = 75 nm) was used to protect the detector from laser light and sensitizer fluorescence. Typical decay times were 28 ± 1 μs, which is characteristic for singlet oxygen decay in toluene.[4] Tetraphenylporphyrin was used as a standard to determine the relative singlet oxygen yield (Φ_Δ).[5] The relative singlet oxygen quantum yield for **5** in air-saturated toluene was 0.72 which is close to the singlet oxygen quantum yield for the nickel-free form of **2** (Φ_Δ = 0.77).[1] No photodegradation as measured by changes in the absorption spectrum of compound **5**, was observed under these conditions. This stability of **5** towards singlet oxygen is surprising. In many porphyrins with appending double bonds the double bond reacts chemically with the singlet oxygen generated by the system. An example is the purpurin obtained by Woodward et al.: During the total synthesis of chlorophyll *a* photo-oxygenation by the singlet oxygen generated by the purpurin derivative itself leads to cleavage of the double bond in the annulated cyclopentene ring.[6] The stability of compound **5** towards singlet oxygen attack on the appending double bond can be explained by the too low electron density of the double bond due to the electron-withdrawing capacity of the nitrile function. The hydrogen atom attached to C2^8 is both allylic and geminal



Scheme 4. Reaction of diphenylmethyl cation with the nickel complex of 5-(2′-cyanovinyl)mesoporphyrin dimethyl ester **1**.

to a tertiary amine function which makes it in general very reactive towards singlet oxygen and other oxidizing agents. However, due to structural restrictions caused by the presence of the *N*-methylaniline group almost perpendicular to the plane of the porphyrin ring, the hydrogen atom at position 2^8 is forced into the plane of the cyclohexadiene ring preventing any stabilization of the species that results from the removal of the hydrogen atom in position 2^8 by the double bond and the aniline function. It is a fortunate coincidence that the precise structure makes compound **5** stable towards attack by singlet oxygen. The nickel complex **4** was investigated in the same way; no singlet oxygen was observed ($\Phi_\Delta < 0.01$).

Biological Effects of 5

First, a toxicity test of **5** in cultures of A549 lung carcinoma cells (American Type Culture Collection) was carried out in the dark. The cells were incubated in a medium containing **5** at various concentrations. The cultures were left overnight in the dark at 37 °C. Using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay based on the methods of Tada[7] and Carmichael[8] the cell survival was obtained as a percentage of untreated cells as control. Even at concentrations up to 30 μg/mL in the medium cell, survival was complete within experimental error, showing that **5** is essentially nontoxic towards the lung carcinoma cells in the dark.

After incubating the lung cancer cells for 4 h with **5** in the dark followed by illumination for 15 min with white light of 30 mW/cm^2, the lung carcinoma cells were killed completely at concentrations of 1 μg/mL and higher. Further experiments in triplet at concentrations lower than 1 μg/mL with **5** as well as with mTHPC[9–11] indicated that cell survival was 30% at concentrations of 0.15 μg/mL for both photosensitizers. This means that in the presence of air and light **5** acts as a very efficient photodynamic agent at much lower concentrations than the quino-annulated porphyrins described before.

Conclusions

The racemic form of the dimethyl ester of 2′-cyano-8′-formyl-*N*′-methyl-1,1a,5a,6-tetrahydroacrido[4,5,5a,6-*bcd*]-annulated 2,3-dihydromesoporphyrin **5** is formed in 22% yield in a one-pot reaction by attack of the Vilsmeier reagent derived from *N*-methylformanilide and phosphorus oxychloride on the α-carbon atom of the acrylonitrile function in [5-(2′-cyanovinyl)mesoporphyrin]nickel(II) complex **1**. This initial attack is followed by a cascade of steps leading to the above-mentioned final product.

The system could easily be separated into the two optically pure forms by separation on a chiral column. Based on the reaction mechanism, it is to be expected that in a similar way using other aromatic acylamide systems a wide range of new systems with analogous structures will be easily available, which can also be separated into pure optical isomers. In order to further investigate the reactivity of the sterically hindered *meso*-acrylonitrile function, we treated **1** with the Vilsmeier reagent obtained from *N*,*N*-dimethylformamide and phosphorus oxychloride, and in this way we obtained two different *peri*-condensed quinoporphyrin systems, which could not be separated into their pure forms thus far. It is clear that the less delocalized positive charge in this carbenium ion attacks the nitrogen function of the *meso*-acrylonitrile group leading to a direct route for the *peri*-condensed quinoporphyrin series without oxidative processes as has been discribed in our earlier publication where the localized hard proton also attacks the nitrogen atom of the acrylonitrile group. In order to test that the hard or soft acid character of the reagent determines whether the primary attack is either on the nitrogen atom of the acrylonitrile group or on the α-carbon atom, we also treated the hindered *meso*-acrylonitrile-substituted compound **1** with the diphenylcarbenium ion. In this case only the (*Z*) and the (*E*) form of {5-[2′-cyano-2′-(diphenylmethyl)vinyl]mesoporphyrin dimethyl ester}nickel(II) complex are formed resulting from the attack of the carbenium ion on the α-carbon atom of the acrylonitrile side chain.

The racemic dimethyl ester of 2′-cyano-8′-formyl-*N*′-methyl-1,1a,5a,6-tetrahydro-acrido[4,5,5a,6-*bcd*]-annulated 2,3-dihydromesoporphyrin **5** is an efficient singlet oxygen generator while it is nontoxic towards lung carcinoma cells. Treatment of the carcinoma cells with the combination of **5**, air and light gives an efficient cell-killing complex at very low concentrations of 0.15 μg/mL of photosensitizer **5**.

We feel that because many similar analogs of **5** can be made available, there should be an optimal starting condition to find a system that has all the required properties that will lead to an optical system for clinical photodynamic therapy.

Experimental Section

General: [5-(2′-Cyanovinyl)mesoporphyrinato]nickel complex **1** was prepared from mesoporphyrin dimethyl ester according to a literature procedure.[1] *N*-Methylformanilide, phosphorus oxychloride, (diethylphosphono)acetonitrile, nickel(II) acetate tetrahydrate and benzhydrol were obtained from Aldrich. Sodium hydride was obtained from Merck. Sulfuric acid was obtained from Boom. Silica gel 60 (230–400 mesh) was obtained from Fluka and *N*,*N*-dimethylformamide, tetrahydrofuran (THF), diethyl ether, dichloromethane, dichloroethane, chloroform, formic acid and methanol were obtained from Biosolve. THF and diethyl ether were distilled prior to use to remove the stabilizer. $CDCl_3$ used for NMR spectroscopy was treated with potassium carbonate to remove traces of acid. Thin layer chromatography (TLC) was accomplished with TLC aluminum sheets covered with silica gel 60, F254 from Merck. NMR spectra were measured with a Bruker DPX300 or a Bruker DMX600. ^{1}H-2D-COSY, ^{1}H-2D-NOESY, Attached Proton Test (APT) ^{13}C NMR, 2D ^{13}C–^{1}H HSQC NMR, 2D ^{13}C–^{1}H HMBC NMR and 2D ^{15}N–^{1}H HMBC NMR spectroscopy were applied to establish the structure of new compounds. 60.8 MHz ^{15}N NMR inverse-detected NMR spectra were derived from 2D ^{15}N–^{1}H HMBC NMR spectra. UV/Vis spectra are measured with a Perkin–

Elmer Lambda-900 UV/Vis/NIR spectrophotometer. CD spectra were recorded using dichloromethane as the solvent with a Jobin Yvon spex CD6 spectrometer using a cylindrical quartz cell (pathlength 0.1 mm). The CD spectra were baseline-corrected and smoothed. Melting points were measured with a Büchi apparatus and are uncorrected. Fast atom bombardment (FAB) mass spectrometry was carried out using a JEOL JMS SX/SX 102A four-sector mass spectrometer coupled to a JEOL MS-MP9021D/UPD system program. Samples were loaded in a matrix solution (3-nitrobenzyl alcohol) on to a stainless steel probe and bombarded with Xenon atoms with an energy of 3 keV. During the high-resolution measurements a resolving power of 10000 (10% valley definition) was used. Electron spray ionization (ESI) mass spectrometry was carried out with a Thermo Finnigan LTQ-FT with a 7 Tesla superconducting magnet. The high-resolution measurements were carried out with a resolving power of 100000. The HPLC experiments were conducted with a Spectroflow 400 solvent delivery system, a Chiralcel OD column, and a Spectroflow 757 absorbance detector set at a wavelength of 254 nm.

Synthesis

(2′-Cyano-8′-formyl-*N*′-methyl-1,1a,5a,6-tetrahydroacrido[4,5,5a,6-*bcd*]-Annulated 2,3-Dihydromesoporphyrin dimethyl ester)nickel Complex (4): $POCl_3$ (0.40 mL, 4.3 mmol) was added dropwise to *N*-methylformanilide (0.55 mL, 4.5 mmol) after which the mixture was stirred for 30 min. This mixture was then added to a solution of 500 mg (0.71 mmol) of [5-(2′-cyanovinyl)mesoporphyrin dimethyl ester]nickel complex **1** in 45 mL of dichloroethane. The reaction was monitored with TLC using a mixture of 1% methanol in dichloromethane as the eluent. After 1 h of stirring at room temperature, the initially brown solution had turned green and 60 mL of a saturated aqueous solution of sodium acetate was added to hydrolyse the product mixture. This mixture was stirred for 16 h after which the organic layer was separated and the solvent evaporated under reduced pressure. *N*-Methylformanilide and *N*-methylaniline in the crude product mixture were removed using silica gel flash chromatography with diethyl ether/hexanes (1:1). The porphyrin mixture was separated using silica gel chromatography with dichloromethane. The first brown fraction contained 160 mg (0.23 mmol, 28%) of the starting compound, the following green bands contained 105 mg (0.14 mmol, 20%) of a mixture of the nickel complexes of *meso*-formylated 5-(2′-cyanovinyl)mesoporphyrin dimethyl ester. The last bright green band contained 130 mg (0.16 mmol, 22%) of the new compound **4**. HR-FAB MS [M + H]: found: *m*/*z* = 847.3133, calcd. for $^{12}C_{48}{}^{1}H_{49}{}^{14}N_{6}{}^{16}O_{5}{}^{58}Ni^{+}$: *m*/*z* = 847.3118. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 0.24 [t, 3 H, ^3J(3$^{1'}$-H–3$^{2'}$-H) = 7.5 Hz, 3$^{2'}$-CH_3], 1.45 (m, 1 H, 3$^{1'}$-CH^a), 1.54 [t, 3 H, ^3J(8^1-H–8^2-H) = 7.6 Hz, 8^2-CH_3], 1.75 (m, 1 H, 3$^{1'}$-CH^a), 2.31 (s, 3 H, 2$^{1'}$-CH_3), 2.93 (m, 2 H, 13^2-CH_2), 3.00 (s, 3 H, 7^1-CH_3), 3.03 (m, 2 H, 17^2-CH_2), 3.06 (s, 3 H, 12^1-CH_3), 3.11 (s, 3 H, 18^1-CH_3), 3.38 (m, 1 H, 8^1-CH^a), 3.45 (m, 1 H, 8^1-CH^b), 3.53 (s, 3 H, 2$^{7'}$-NCH_3), 3.66 (s, 3 H, 13^5-OCH_3), 3.72 (s, 3 H, 17^5-OCH_3), 3.85 (m, 2 H, 13^1-CH_2), 3.90 (m, 2 H, 17^1-CH_2), 4.61 (s, 1 H, 2^8-CH), 6.61 [d, ^3J(2^4-H–2^5-H) = 8.8 Hz, 1 H, 2^5-H], 7.09 [d, ^4J(2^2-H–2^4-H) = 1.7 Hz, 1 H, 2^2-H], 7.36 [dd, ^3J(2^4-H–2^5-H) = 8.8, ^4J(2^2-H–2^4-H) = 1.7 Hz, 1 H, 2^4-H], 8.29 (s, 1 H, 20-CH), 8.60 (s, 1 H, 2^{10}-CH), 8.86 (s, 1 H, 10-CH), 8.97 (s, 1 H, 15-CH), 9.16 (s, 2$^{3'}$-CHO) ppm. ^{13}C NMR (150.9 MHz): δ = 9.5 (CH_3-3$^{2'}$), 10.9 (CH_3-12^1), 11.2 (CH_3-18^1), 14.9 (CH_3-7^1), 17.2 (CH_3-8^2), 19.3 (CH_2-8^1), 21.2 (CH_2-13^1), 21.3 (CH_2-17^1), 21.4 (CH_3-2$^{1'}$), 27.0 (CH_3-3$^{1'}$), 36.4 (CH_2-13^2), 36.4 (CH_2-17^2), 37.0 (NCH_3-2$^{7'}$), 51.7 (OCH_3-13^5), 51.8 (OCH_3-17^5), 53.7 (C-3), 54.0 (C-2), 58.7 (CH-2^8), 94.9 (CH-20), 99.7 (CN-2$^{9'}$), 100.3 (C-5), 103.5 (CH-15), 104.3 (CH-10), 110.4 (CH-2^5), 121.9 (C-2^9), 125.4 (C-2^1), 125.8 (C-2^3), 127.8 (CH-2^2), 130.5 (C-7), 130.9 (CH-2^4), 133.7 (C-18), 134.8 (C-12), 136.1 (C-9), 137.2 (C-16), 137.6 (C-13), 138.6 (C-14), 139.7 (C-11), 141.7 (C-17), 143.4 (C-2^{10}), 143.8 (C-6), 146.4 (C-19), 147.9 (C-8), 149.0 (C-4), 149.5 (C-2^6), 156.0 (C-1), 173.3 (C-13^3), 173.4 (C-17^3), 189.8 (CH-2$^{3'}$) ppm. UV/Vis (CH_2Cl_2): λ_{max} (log ε) = 339 (4.5), 428 (4.8), 511 (3.7), 648 (4.4) nm.

[5-(2′-Cyanovinyl)-20-formylmesoporphyrin dimethyl ester]nickel Complex 6a: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.59 [t, ^3J(3^1-H–3^2-H) = 7.6 Hz, 3^2-CH_3 and 8^2-CH_3], 3.01 [t, ^3J(13^1-H–13^2-H) = 7.4 Hz, 13^2-CH_2 and 17^2-CH_2], 3.22 (s, 12 H, 2^1-CH_3, 7^1-CH_3, 12^1-CH_3 and 18^1-CH_3), 3.59 [q, ^3J(3^1-H–3^2-H) = 7.6 Hz, 3^1-CH_2 and 8^1-CH_2], 3.68 (2 s, 6 H, 13^5-OCH_3 and 17^5-OCH_3), 3.96 [t, ^3J(13^1-H–13^2-H) = 7.4 Hz, 13^1-CH_2 and 17^1-CH_2], 9.46 [d, 1 H, ^3J(5^1-H–5^2-H) = 16.1 Hz, 5^1-CH], 11.80 (s, 1 H, 20-CHO). UV/Vis (CH_2Cl_2): λ_{max} (log ε) = 328 (4.2), 440 (4.9), 607 (sh, 3.9), 653 (4.0) nm.

2′-Cyano-8′-formyl-*N*′-methyl-1,1a,5a,6-tetrahydroacrido[4,5,5a,6-*bcd*]-Annulated 2,3-Dihydromesoporphyrin Dimethyl Ester 5: Complex **4** (50 mg, 0.06 mmol) was dissolved in 4.0 mL of concentrated sulfuric acid and stirred at room temperature for 30 min. The reaction mixture was neutralized with an aqueous solution of sodium acetate and the precipitate was filtered off and washed with water. The crude product was purified by silica gel chromatography using a mixture of 1% of methanol in dichloromethane as the eluent. The product was recrystallized from a mixture of dichloromethane and hexanes giving a yield of 29 mg (0.04 mmol, 62%). HR-FAB MS [M + H]: found: *m*/*z* = 791.3931, calcd. for $^{12}C_{48}{}^{1}H_{51}{}^{14}N_{6}{}^{16}O_{5}{}^{+}$: *m*/*z* = 791.3921. ^{1}H NMR (600 MHz, $CDCl_3$): δ = –1.12 (br., 1 H, NH), –0.76 (br., 1 H, NH), 0.07 [t, 3 H, ^3J(3$^{1'}$-H–3$^{2'}$-H) = 7.2 Hz, 3$^{2'}$-CH_3], 1.65 (m, 1 H, 3$^{1'}$-CH^a), 1.67 [t, 3 H, ^3J(8^1-H–8^2-H) = 7.7 Hz, 8^2-CH_3], 1.91 (m, 1 H, 3$^{1'}$-CH^a), 2.64 (s, 3 H, 2$^{1'}$-CH_3), 3.08 [t, ^{3}J(13^1-H–13^2-H) = 7.7 Hz, 2 H, 13^2-CH_2], 3.20 [t, ^3J(17^1-H–17^2-H) = 7.7 Hz, 2 H, 17^2-CH_2], 3.28 (s, 3 H, 12^1-CH_3), 3.43 (s, 3 H, 7^1-CH_3), 3.50 (s, 3 H, 18^1-CH_3), 3.56 (s, 3 H, 2$^{7'}$-NCH_3), 3.65 (s, 3 H, 13^5-OCH_3), 3.70 (s, 3 H, 17^5-OCH_3), 3.81 (m, 1 H, 8^1-CH^a), 3.86 (m, 1 H, 8^1-CH^b), 4.06 (m, 2 H, 13^1-CH_2), 4.24 [t, ^3J(17^1-H–17^2-H) = 7.7 Hz 2 H, 17^1-CH_2], 4.82 (s, 1 H, 2^8-CH), 6.49 [d, ^3J(2^4-H–2^5-H) = 8.7 Hz, 1 H, 2^5-H], 7.21 [dd, ^3J(2^4-H–2^5-H) = 8.7, ^4J(2^2-H–2^4-H) = 1.3 Hz, 1 H, 2^4-H], 7.59 (br., 1 H, 2^2-H), 9.04 (s, 1 H, 20-CH), 9.14 (s, 2$^{3'}$-CHO), 9.40 (s, 1 H, 2^{10}-CH), 9.53 (s, 1 H, 10-CH), 9.57 (s, 1 H, 15-CH) ppm. ^{13}C NMR (150.9 MHz): δ = 9.9 (CH_3-3$^{2'}$), 11.3 (CH_3-18^1), 11.5 (CH_3-12^1), 15.5 (CH_3-7^1), 17.2 (CH_3-8^2), 19.3 (CH_2-8^1), 21.4 (CH_2-17^1), 21.6 (CH_2-13^1), 22.5 (CH_3-2$^{1'}$), 27.7 (CH_3-3$^{1'}$), 36.4 (CH_2-17^2), 36.8 (CH_2-13^2), 37.5 (NCH_3-2$^{7'}$), 51.7 (OCH_3-13^5), 51.8 (OCH_3-17^5), 53.6 (C-3), 54.3 (C-2), 58.0 (CH-2^8), 94.3 (CH-20), 100.5 (CN-2$^{9'}$), 101.2 (C-5), 102.8 (CH-15), 102.9 (CH-10), 110.8 (CH-2^5), 122.7 (C-2^9), 125.4 (C-2^3), 126.3 (C-2^1), 126.7 (C-7), 128.7 (CH-2^4), 130.0 (C-18), 130.8 (CH-2^4), 131.1 (C-9), 133.0 (C-16), 137.6 (C-17), 137.9 (C-6), 138.2 (C-12), 139.6 (C-19), 140.2 (C-13), 143.8 (C-8), 144.4 (C-2^{10}), 149.2 (C-2^6), 150.8 (C-14), 151.0 (C-11), 161.9 (C-4), 167.8 (C-1), 173.6 (C-13^3), 173.3 (C-17^3), 189.6 (CH-2$^{3'}$) ppm. UV/Vis (CH_2Cl_2): λ_{max} (log ε) = 342 (4.7), 428 (5.1), 518 (3.9), 560 (4.3), 633 (3.8), 688 (4.6) nm.

Reaction of 1 with (Chloromethylene)dimethylammonium Dichlorophosphate: $POCl_3$ (0.40 mL, 4.3 mmol) was added dropwise to an ice-cold mixture of dimethylformamide (0.55 mL, 4.5 mmol) and 1.0 mL of chloroform, after which the mixture was stirred at room temperature for 20 min. This mixture was then added to a solution of [5-(2′-cyanovinyl)mesoporphyrin dimethyl ester]nickel complex **1** (500 mg, 0.71 mmol) in 20 mL of chloroform. The reaction was monitored by TLC using a mixture of 1% methanol in dichloro-

methane as the eluent. After 16 h of stirring at room temperature, an aqueous solution of sodium acetate was added until the pH was 7 and the reaction mixture was stirred until hydrolysis was complete. The chloroform was then removed by distillation under reduced pressure, the solid mixture obtained in this way was filtered off and dissolved in dichloromethane. With silica gel flash chromatography a complex mixture of formylated products (350 mg, 0.48 mmol, 67%) was obtained using 2% methanol in dichloromethane as the eluent. When using 5% methanol in dichloromethane, a green fraction was collected which contained 45 mg of an impure mixture containing quino-annulated porphyrins as recognized from the V-shaped spot on the TLC obtained with 80%THF and 20% diethyl ether (v/v). This latter fraction was purified on silica gel for a second time using a mixture of 60% THF and 40% diethyl ether (v/v). This yielded 25 mg of a mixture containing **7** and **8** in a 2:1 ratio.

(3-Deethyl-2-demethylquino[4,4a,5,6-*bcd*]mesoporphyrin dimethyl ester)nickel Complex 7: HR-FAB MS [M + H]: found: *m*/*z* = 684.2134, calcd. for $^{12}C_{38}{}^{1}H_{36}{}^{14}N_5{}^{16}O_4{}^{58}Ni^+$: *m*/*z* = 684.2121. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.53 [t, 3 H, $^3J(8^1\text{-H}–8^2\text{-H})$ = 7.9 Hz, 8^2-CH_3], 2.79 (s, 3 H, 18^1-CH_3), 2.91–2.97 (m, 2 H, 17^2-CH_2), 2.99 (s, 3 H, 7^1-CH_3), 3.04–3.09 (m, 2 H, 13^2-CH_2), 3.37 (s, 3 H, 12^1-CH_3), 3.39 [q, $^3J(8^1\text{-H}–8^2\text{-H})$ = 7.9 Hz, 8^1-CH_2], 3.70 and 3.73 (2 s, 6 H, 2 OCH_3), 3.83 (m, 2 H, 17^1-CH_2), 4.03 (m, 2 H, 13^1-CH_2), 7.90 [d, 1 H, $^3J(2^5\text{-H}–2^6\text{-H})$ = 9.3 Hz, 2^5-CH], 8.17 [d, 1 H, $^3J(2^1\text{-H}–2^2\text{-H})$ = 4.6 Hz, 2^1-CH], 8.58 (s, 1 H, 20-CH), 8.91 [d, 1 H, $^3J(2^5\text{-H}–2^6\text{-H})$ = 9.3 Hz, 2^6-CH], 8.98 (s, 1 H, 10-CH), 9.03 (s, 1 H, 15-H), 9.31 (s, 1 H, 2^2-CH) ppm.

(8-Deethyl-7-demethyl-3′-methylquino[4,4a,5,6-*efg*]mesoporphyrin dimethyl ester)nickel Complex 8: HR-FAB MS [M + H]: found: *m*/*z* = 698.2283, calcd. for $^{12}C_{39}{}^{1}H_{38}{}^{14}N_5{}^{16}O_4{}^{58}Ni^+$: *m*/*z* = 698.2277. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.59 [t, 3 H, $^3J(3^1\text{-H}–3^2\text{-H})$ = 7.7 Hz, 3^2-CH_3], 2.71 (s, 3 H, 12^1-CH_3), 2.87 (s, 3 H, $5^{6\prime}$-CH), 2.91–2.97 (m, 2 H, 13^2-CH_2), 3.04–3.09 (m, 2 H, 17^2-CH_2), 3.12 (s, 3 H, 2^1-CH_3), 3.31 (s, 3 H, 18^1-CH_3), 3.55 [q, $^3J(3^1\text{-H}–3^2\text{-H})$ = 7.7 Hz, 3^1-CH_2], 3.68 and 3.71 (2 s, 6 H, 2 OCH_3), 3.83 (m, 2 H, 13^1-CH_2), 4.03 (m, 2 H, 17^1-CH_2), 8.08 [d, 1 H, $^3J(5^1\text{-H}–5^2\text{-H})$ = 9.3 Hz, 5^2-CH], 8.40 (s, 1 H, 10-CH), 9.03 (s, 1 H, 5^5-CH), 9.05 [d, 1 H, $^3J(5^1\text{-H}–5^2\text{-H})$ = 9.3 Hz, 5^1-CH], 9.11 (s, 1 H, 15-CH and 20-CH) ppm.

Reaction of 1 with Diphenylmethyl Carbenium Ion Generated from Benzhydrol in Formic Acid: A mixture of **1** (200 mg, 0.28 mmol) and benzhydrol (51 mg, 0.28 mmol) in 5.0 mL of formic acid was refluxed for 2 min after which the mixture was poured into 200 mL of saturated sodium carbonate. The crude product was extracted with dichloromethane and dried with magnesium sulfate after which the solvent was evaporated. The crude product was purified on silica gel using a mixture of diethyl ether and hexanes (35:65, v/v). Two red fractions were obtained: The first red fraction was recrystallized yielding 45 mg (0.05 mmol, 18%) of (*E*)-**9**; the second brown-red band was also recrystallized yielding 72 mg of (*Z*)-**9** (0.08 mmol, 29%).

{(*E*)-[2′-Cyano-2′-(diphenylmethyl)vinyl]mesoporphyrin dimethyl ester}nickel Complex (*E*)-9: HR-FAB MS [M + H]: found: *m*/*z* = 868.3374, calcd. for $^{12}C_{52}{}^{1}H_{52}{}^{14}N_5{}^{16}O_4Ni^+$: *m*/*z* = 868.3373. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.63 [2 t, 6 H, $^3J(3^1\text{-H}–3^2\text{-H})$ = 7.5 Hz, 3^2-CH_3 and 8^2-CH_3}, 2.65 (s, 1 H, 5^3-CH), 3.06 (s, 3 H, 7^1-CH_3), 3.12–3.19 (m, 13^2-CH_2 and 17^2-CH_2), 3.31 (s, 3 H, 2^1-CH_3), 3.42 and 3.44 (2 s, 6 H, 12^1-CH_3 and 18^1-CH_3), 3.59 (m, 1 H, 3^{1a}-CH), 3.70 and 3.71 (2 s, 6 H, 13^5-OCH_3 and 17^5-OCH_3), 3.69–3.74 (m, 2 H, 8^1-CH_2), 3.82 (m, 1 H, 3^{1a}-CH), 4.16 (m, 13^{1a}-CH_2 and 17^{1a}-CH_2), 4.23 (m, 13^{1b}-CH_2 and 17^{1b}-CH_2), 6.11 [d, 2 H, ^3J(*ortho*-H–*meta*-H) = 7.5 Hz, $Ph1^{ortho}$-CH], 6.17 [d, 2 H, ^3J(*ortho*-H–*meta*-H) = 7.5 Hz, $Ph2^{ortho}$-CH], 6.79 [dd, 2 H, ^3J(*ortho*-H–*meta*-H) = 7.5, ^3J(*meta*-H–*para*-H) = 7.5 Hz, $Ph2^{meta}$-CH], 6.83 [dd, 2 H, ^3J(*ortho*-H–*meta*-H) = 7.5, ^3J(*meta*-H–*para*-H) = 7.5 Hz, $Ph1^{meta}$-CH], 6.89 [t, 1 H, ^3J(*meta*-H–*para*-H) = 7.5 Hz, $Ph2^{para}$-CH], 6.97 [t, 1 H, ^3J(*meta*-H–*para*-H) = 7.5 Hz, $Ph1^{para}$-CH], 9.56 (2 s, 2 H, 10-CH and 20-CH), 9.60 (s, 1 H, 15-CH), 9.75 (s, 1 H, 5^1-CH) ppm. ^{13}C NMR (150.9 MHz): δ = 11.4 (CH_3-2^1), 11.5 (2 signals CH_3-12^1 and CH_3-18^1), 16.3 (2 signals, CH_3-3^2 and CH_3-7^1), 17.4 (CH_3-8^2), 19.5 (CH_2-8^1), 21.6 (2 signals, CH_2-13^2 and CH_2-17^2), 22.1 (CH_2-3^1), 36.6 and 36.7 (CH_2-13^2 and CH_2-17^2), 48.7 (CH-5^1), 51.8 (OCH_3-13^5 and OCH_3-17^5), 96.7 (CH-15), 97.4 and 97.5 (CH-10 and CH-20), 104.2, 119.5, 123.4, 126.6 (CH-$Ph2^{para}$), 126.8 (CH-$Ph1^{para}$), 127.8 (CH-$Ph2^{ortho}$), 127.9 (CH-$Ph2^{meta}$), 128.0 (CH-$Ph1^{meta}$), 128.1 (CH-$Ph1^{ortho}$), 137.3, 137.7, 137.8, 137.8, 138.6, 138.8, 139.3, 139.4, 139.5, 140.2, 140.4, 140.5, 141.0, 144.3, 145.9, 147.1 (CH-5^1), 173.5, 173.5 ppm. UV/Vis (CH_2Cl_2): λ_{max} (log ε) = 293 (4.1), 346 (4.1), 405 (5.1), 530 (4.0), 565 (4.2), 649 (3.4) nm.

{(*Z*)-[2′-Cyano-2′-(diphenylmethyl)vinyl]mesoporphyrin dimethyl ester}nickel Complex (*Z*)-9: HR-FAB MS [M + H]: found: *m*/*z* = 868.3362, calcd. for $^{12}C_{52}{}^{1}H_{52}{}^{14}N_5{}^{16}O_4Ni^+$: *m*/*z* = 868.3373. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.46 [t, 3 H, $^3J(3^1\text{-H}–3^2\text{-H})$ = 7.5 Hz, 3^2-CH_3], 1.62 [t, 3 H, $^3J(8^1\text{-H}–8^2\text{-H})$ = 7.7 Hz, 8^2-CH_3], 3.09 (m, 4 H, 13^2-CH_2 and 17^2-CH_2), 3.12 (s, 3 H, 7^1-CH_3), 3.32 (s, 3 H, 2^1-CH_3), 3.38 (2 s, 3 H, 12^1-CH_3 and 18^1-CH_3), 3.57 (m, 1 H, 3^1a-CH), 3.62 (m, 1 H, 3^1b-CH), 3.68 and 3.69 (2 s, 6 H, 2 OCH_3), 3.73 (m, 1 H, 8^1a-CH), 3.82 (m, 1 H, 8^1b-CH), 4.10 (m, 2 H, 13^1a-CH and 17^1a-CH), 4.20 (m, 1 H, 13^1b-CH and 17^1b-CH), 5.33 [d, 1 H, $^4J(5^1\text{-H}–5^3\text{-H})$ = 1.1 Hz, 5^3-H], 7.29–7.41 (m, 10 H, phenyl-H), 9.07 [d, 1 H, $^4J(5^1\text{-H}–5^3\text{-H})$ = 1.1 Hz, 5^1-H], 9.46 (s, 3 H, 10-CH, 15-CH and 20-CH) ppm. ^{13}C NMR (150.9 MHz): δ = 11.4 and 11.4 (2^1-CH_3, 12^1-CH_3 and 18^1-CH_3), 16.4 (8^2-CH_3), 17.1 (7^1-CH_3), 17.3 (3^2-CH_3), 19.6 (8^1-CH_2), 21.6 (13^1-CH_2 and 17^1-CH_2), 22.3 (3^1-CH_2), 36.6 (13^2-CH_2 and 17^2-CH_2), 49.1 (OCH_3), 51.8 (OCH_3), 56.8 (5^3-CH), 96.8, 97.5, 97.5, 105.4, 116.1, 125.3, 127.7(Ph-CH), 129.0 (Ph-CH), 129.1(Ph-CH), 136.6, 137.7, 138.0, 138.9, 139.5, 139.7, 139.9, 140.1, 140.1, 140.8, 141.0, 143.9, 146.3, 147.0 (5^1-CH), 173.5, 173.5 ppm. UV/Vis (CH_2Cl_2): λ_{max} (log ε) = 344 (4.1), 408 (4.9), 530 (4.0), 569 (4.0), 651 (3.3) nm.

Acknowledgments

This investigation has been financially supported by the Dutch Technology Foundation (STW) and by the Netherlands Research Council for Chemical Sciences (CW). The authors wish to thank Harimex and Sato Pharmaceuticals for providing protoporphyrin dimethyl ester. The authors also wish to thank Ruth Edge for setting up the equipment for the singlet oxygen measurements, Han Peeters for recording the FAB high-resolution mass spectra, Rian van den Nieuwendijk for conducting HPLC experiments and Wim Jesse for recording CD spectra.

[1] R. N. S. van der Haas, R. L. P. de Jong, M. Noushazar, K. Erkelens, T. G. M. Smijs, Y. Liu, P. Gast, H. J. Schuitmaker, J. Lugtenburg, *Eur. J. Org. Chem.* **2004**, *19*, 4024–4038.
[2] O. Meth-Cohn, B. Tarnowski, *Adv. Heterocyc. Chem.* **1982**, *31*, 207–236.
[3] G. C. Gullickson, D. E. Lewis, *Synthesis* **2003**, 681–684.
[4] M. Okamoto, F. Tananka, *J. Phys. Chem.* **1993**, *97*, 177–180.
[5] F. Wilkinson, W. P. Helman, A. B. Ross, *J. Phys. Chem. Ref. Data* **1993**, *22*, 113–262.
[6] R. B. Woodward, W. A. Ayer, J. M. Beaton, F. Bickelhaupt, R. Bonnet, P. Buchschacher, G. L. Closs, H. Dutler, J. Hannah,

F. P. Hauck, S. Itô, A. Langemann, E. Le Goff, W. Leimgruber, W. Lwowski, J. Sauer, Z. Valenta, H. Volz, *J. Am. Chem. Soc.* **1960**, *82*, 3800–3802.
[7] H. Tada, O. Shiho, *J. Immunol. Methods* **1986**, *93*, 157–165.
[8] J. Carmichael, W. G. de Graaff, *Cancer Res.* **1987**, *47*, 936–942.
[9] *meso*-Tetrahydroxyphenylchlorin, obtained from Scotia, unpublished results.
[10] R. Bonnett, *Chemical Aspects of Photodynamic Therapy*, Gordon & Breach Scientific Publishers, Amsterdam, **2000**, p. 256.
[11] R. K. Pandey, G. Zheng, *Porphyrin Handbook* (Ed.: K. M. Kadish, K. M. Smith, R. Guilard), Academic Press, San Diego, **2000**, vol. 6, chapter 43, p. 158–159, 214–224.

Received: March 9, 2005
Published Online: July 12, 2005

