

1,3-Dipolar cycloaddition of nitrile imines to *meso*-tetraarylporphyrins

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Dedicated to Prof. António Rocha Gonçalves
on the occasion of his 70th anniversary

Abstract

N-Aryl-*C*-ethoxycarbonylnitrile imines react with *meso*-tetrakis(pentafluorophenyl)porphyrin in 1,3-dipolar cycloadditions to yield novel pyrazolochlorins in moderate yields. The nitrile imines were generated *in situ* by base-induced dehydrobromination of ethyl hydrazono- α -bromoglyoxylates. A number of different experimental conditions were considered for these cycloadditions, namely different bases, solvents and temperature; the best results were obtained using potassium carbonate in refluxing toluene. The photophysical properties of the new chlorins were investigated and the results suggest that two of them have potential for use in photodynamic therapy.

Keywords: 1,3-Dipolar cycloaddition, nitrile imines, porphyrins, pyrazoline derivatives, singlet oxygen, fluorescence

Introduction

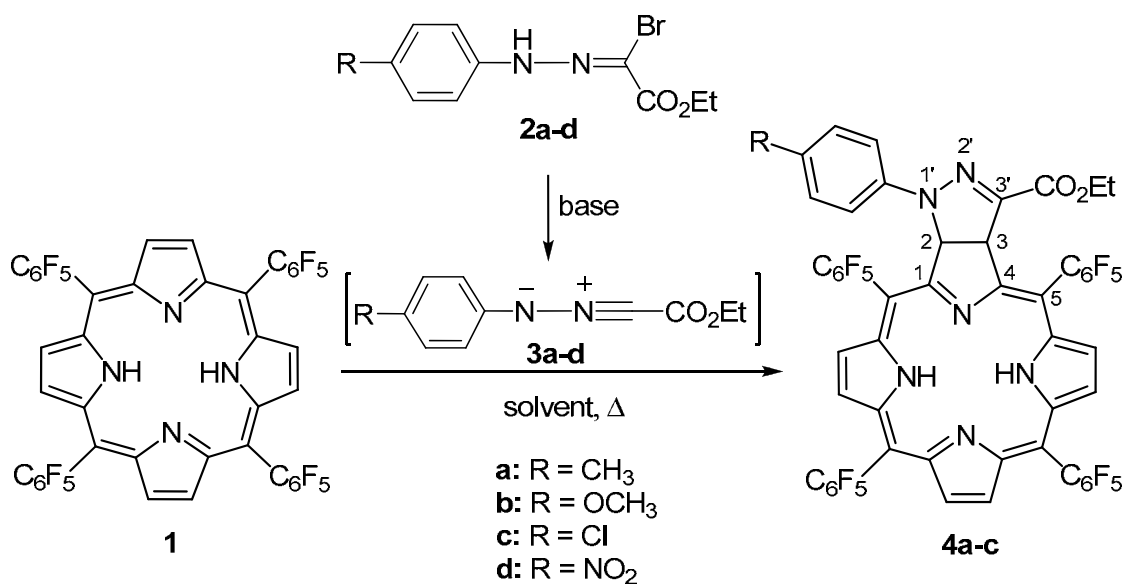
The growing interest in tetrapyrrolic macrocycles is due to their physicochemical features that strongly encourage their application in several fields; indeed, porphyrins are used as catalysts, advanced biomimetic models for photosynthesis, new electronic materials, sensors and drugs.¹ In the biomedical field, porphyrins and related species are successfully being employed for the

treatment of several diseases by photodynamic therapy (PDT),^{2,3} a technique which relies on the administration of a photosensitizing agent and subsequent irradiation with light of appropriate wavelength. In several countries, certain neoplastic diseases are being treated by PDT, using a formulation containing a purified derivative of hematoporphyrin (Photofrin[®]). Bactericidal and virucidal activities have also been reported for some porphyrin derivatives.⁴ Also, over the last few years, age-related macular degeneration is also being treated using a chlorin (a dihydroporphyrin) derivative.⁵ Other derivatives, like bacteriochlorins (tetrahydroporphyrins), are being tested in PDT. The spectroscopic features of chlorins and bacteriochlorins (in particular their enhanced absorption in the 620-750 nm zone of the UV-Vis spectrum) make them appealing potential candidates for PDT. A useful tool to obtain such compounds is the chemical modification of simple porphyrins, namely *via* cycloaddition reactions.⁶ The dipolarophile behaviour of the porphyrin macrocycle has been well documented; we have demonstrated that porphyrins can participate in 1,3-dipolar cycloaddition reactions with a variety of dipoles, such as azomethine ylides,⁷ diazoalkanes,⁸ and nitrones.⁹ Other groups have extended these reactions to carbonyl ylides¹⁰ and to nitrile oxides.¹¹ These reactions lead to functionalized porphyrin, chlorin, bacteriochlorin and isobacteriochlorin derivatives in good yields. In this paper we describe the reaction of a porphyrin with a new class of 1,3-dipoles: the nitrile imines. Nitrile imines have been extensively used to synthesize 4,5-dihydropyrazoles and pyrazoles,^{12,13} compounds which have interesting chemical reactivity, a broad spectrum of biological activity, and a variety of industrial applications.¹⁴ Reactions of porphyrins with nitrile imines can then lead to new compounds containing the chlorin and the pyrazole moieties. Such new products might be considered as potential candidates for biological evaluations.

Results and Discussion

Following our interest in studying the behavior of porphyrins under cycloaddition conditions, we undertook a study of the 1,3-dipolar cycloaddition of porphyrins with several nitrile imine precursors. To the best of our knowledge, such a type of reaction had not been examined so far. The results of such studies are reported here.

Because of the intrinsic instability of nitrile imines, the cycloaddition reactions were performed by *in situ* generation of the 1,3-dipole from hydrazoneyl halides and a base in the presence of the dipolarophile.¹⁵ Nitrile imine precursors bearing different *p*-substituents on the aryl ring were used, aiming to investigate the effect of their electron-withdrawing or -donating effects on the course of the reaction. Several reaction conditions, including different temperatures, solvents and bases were studied. *Meso*-tetrakis(pentafluorophenyl)porphyrin **1** (Scheme 1) was used as the dipolarophile because it shows enhanced reactivity towards 1,3-dipoles, when compared to other porphyrins. Such reactivity is ascribed to the strong electron-withdrawing effect of the pentafluorophenyl groups. However, in the present case it was soon realized that the reactivity of nitrile imines **3** towards porphyrin **1** is not very high (Table 1).



Scheme 1

Table 1. Experimental conditions for the cycloaddition reactions

Entry	Ylide Precursor	Solvent	Base	Oil Bath (°C)	Chlorin Yield % ^a
1	2a	CHCl ₃	NEt ₃	80	-
2	2a	1,4-dioxane	K ₂ CO ₃	100	8
3	2a	toluene	NEt ₃	100	5
4	2a	toluene	K ₂ CO ₃	80	16
5	2a	toluene	K ₂ CO ₃	120	37
6	2a	toluene	Cs ₂ CO ₃	120	24
7	2a	PhCl	K ₂ CO ₃	120	14
8	2a	PhCl	Cs ₂ CO ₃	120	29
9	2a	PhCl	K ₂ CO ₃	140	4
10	2b	1,4-dioxane	K ₂ CO ₃	100	-
11	2b	toluene	NEt ₃	100	-
12	2b	toluene	K ₂ CO ₃	120	40
13	2b	toluene	Cs ₂ CO ₃	120	11
14	2b	PhCl	K ₂ CO ₃	120	16
15	2b	PhCl	Cs ₂ CO ₃	120	28
16	2b	PhCl	K ₂ CO ₃	140	6
17	2c	toluene	K ₂ CO ₃	120	22
18	2c	toluene	Cs ₂ CO ₃	120	17
19	2c	PhCl	K ₂ CO ₃	120	18
20	2c	PhCl	Cs ₂ CO ₃	120	19

^aCalculation based on the porphyrin consumed.

As shown in Table 1, best results were obtained with nitrile imines bearing electron-donating groups in the aryl ring (**3a** and **3b**). In the reaction of porphyrin **1** with the nitrile imine precursor **2d**, under different reaction conditions, no cycloadduct was formed, the starting porphyrin being recovered. The cycloaddition reactions were carried out under various reaction conditions, namely by changing the solvent, base and temperature. Triethylamine, which is typically the base of choice for cycloadditions of nitrile imines,¹⁶⁻²⁰ proved to be unsuitable because its boiling point is considerably lower than the temperature at which the cycloaddition takes place. The use of pyridine, DBU or Ag₂CO₃ did not improve the product yields. The best results were obtained using K₂CO₃ as a base (in refluxing toluene). The use of polar solvents, like 1,4-dioxane (entry 2), did not improve the efficiency of these reactions. It was observed that the yields are lower when the reactions are carried out at 140 °C (entries 9 and 16); this is due to the decomposition of the chlorin at this temperature. In order to improve the yields of the cycloaddition reactions, the use of microwave irradiation²¹ was also considered but no significant improvement was observed.

The low yields observed in the reaction of porphyrin **1** with nitrile imines **3** are probably due to the tendency of these 1,3-dipoles to dimerize, forming bis-diazoethylenes and head-to-tail dimers.²² This chemical behavior of nitrile imines can be rationalised by considering their high carbene character.²³

The structural elucidation of chlorins **4a-c** was based on their UV-Vis, NMR and mass spectra. The UV-Vis spectra of the three new compounds (Figure 1) are very different from that of the starting porphyrin **1**. Compounds **4a-c** show similar UV-Vis spectra, with a sharp peak at *ca.* 650 nm, which is characteristic of chlorin derivatives. The Soret band is centered at 408 nm with a 5 nm hypsochromic shift when compared to that of **1**. Their ¹H NMR spectra showed resonances due to the six aromatic β-protons at above 8 ppm, while the resonances due to protons H-2 and H-3 appeared as two doublets at 6.50-6.70 and 7.40-7.70 ppm, exhibiting a coupling constant *ca.* 9 Hz indicating that they are in a *cis* configuration.²⁴ The high chemical shift for these two doublets is probably due to the deshielding anisotropic effect of the porphyrin macrocycle combined with the proximity of the nitrogen atom and to the anisotropic effect of the ester group. The resonances of the protons of the *p*-substituted *N*-aryl group appear as two doublets between 6.50 and 8.00 ppm. As expected, the singlets generated by the inner pyrrolic protons appear between -1.60 and -1.70 ppm.

Considering the potential application of the new chlorins in PDT, we determined their fluorescence quantum yields and evaluated their ability to generate singlet oxygen. The steady-state fluorescence spectra were measured in toluene solutions under normal air conditions (OD 0.05 at the excitation wavelength 532 nm). The fluorescence quantum yields (Φ_f) of the chlorins were calculated by comparison of the area below the corrected emission spectrum with that of *meso*-tetraphenylporphyrin (TPP). TPP was used as fluorescence standard ($\lambda_{exc} = 532$ nm) with $\Phi_f = 0.11$ in toluene.²⁵ The fluorescence emission spectra of chlorins **4a-c** in toluene are characterized by a strong emission band with a maximum intensity at 664.5 nm for chlorins **4a**

and **4b** and at 662 nm for chlorin **4c** (Figure 1 inset). The fluorescence quantum yields (Φ_f) are summarized in Table 2; chlorin **4c** showed the highest fluorescence quantum yield ($\Phi_f = 0.17$).

Photodynamic therapy (PDT) makes use of the photodynamic effect in which singlet oxygen (1O_2) is generated in the target tissue *via* energy transfer from the first excited triplet state of the photosensitizers (PS) to molecular oxygen in its triplet ground state (3O_2). Today, it is well accepted that 1O_2 plays a key role in both the apoptotic and necrotic pathways of cell death induced by PDT. For this reason, we investigated the photosensitized singlet oxygen generation of the new compounds as one important parameter in order to decide if these dyes would be suitable for use as photosensitizers in PDT. The singlet oxygen luminescence decay was measured at 1270 nm and the quantum yields (Φ_Δ) were calculated according to Equation 1 (AUC is the integrated area under the 1O_2 emission spectrum and Abs is the absorbance at the excitation wavelength). *Meso*-tetraphenylporphyrin (TPP) in toluene was used as reference ($\Phi_\Delta = 0.68$).²⁶ The chlorin samples in toluene and the reference were excited at 420 nm ($OD_{420\text{ nm}} = 0.1$). As can be seen from Table 2, all the synthesized chlorins are able to generate singlet oxygen. Chlorins **4a** and **4c** gave higher singlet oxygen quantum yields ($\Phi_\Delta > 0.68$) than TPP and surprisingly the singlet oxygen quantum yield of derivative **4b** was reduced by a factor of 3 relative to the other two chlorins. The different behavior of compound **4b** is under investigation.

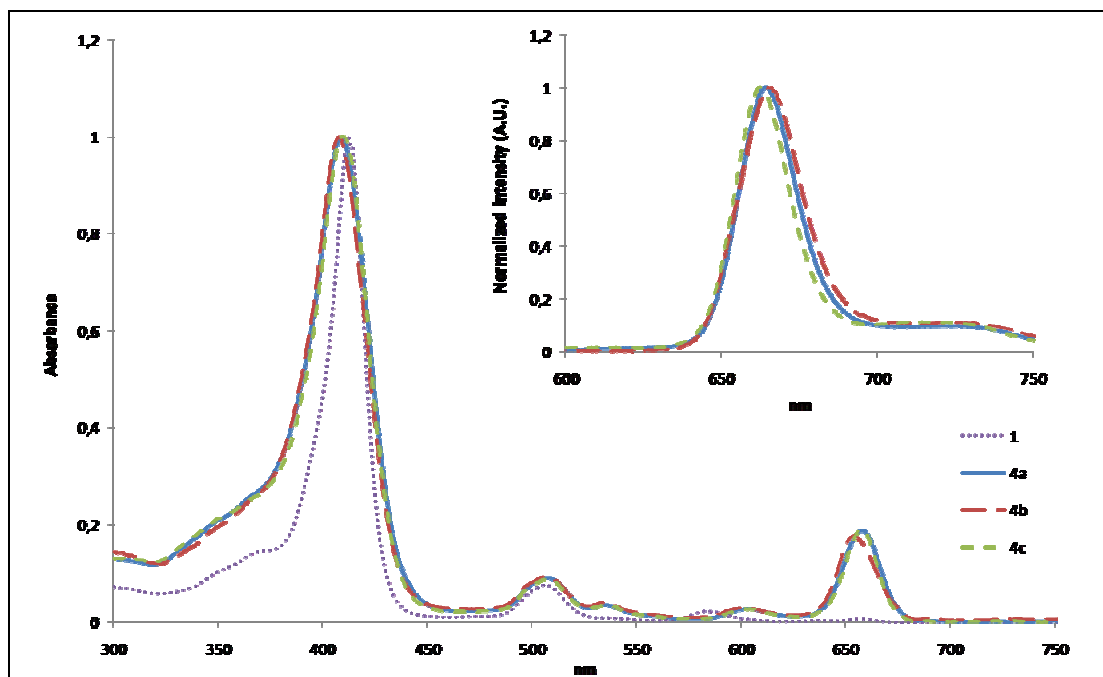


Figure 1. Normalized UV-Vis spectra of compounds **1**, **4a**, **4b** and **4c** in $CHCl_3$. Inset: Normalized fluorescence spectra of **4a-c** in toluene ($\lambda_{exc} = 532$ nm).

Table 2. Fluorescence (Φ_{fl}) and singlet oxygen (Φ_{Δ}) quantum yields of chlorins **4a-c** in toluene

Chlorins	4a	4b	4c
^a Φ_{fl}	0.12	0.08	0.17
^b Φ_{Δ}	0.73	0.23	0.75

^a Optical density of all samples was 0.05 at $\lambda_{exc} = 532$ nm.

^b Optical density of all samples was 0.1 at $\lambda_{exc} = 420$ nm.

$$\Phi_{\Delta}^{sample} = \Phi_{\Delta}^{ref} \frac{AUC^{sample} (1 - 10^{-Abs_{ref}})}{AUC^{ref} (1 - 10^{-Abs_{sample}})} \quad (1)$$

Conclusions

The 1,3-dipolar cycloaddition reactions of nitrile imines with *meso*-tetrakis(pentafluorophenyl)porphyrin **1** were studied under different reaction conditions. The resulting pyrazolochlorin derivatives **4a**, **4b** and **4c** were obtained in acceptable yields (37%, 40% and 22%, respectively) using K_2CO_3 in refluxing toluene. The characteristic absorption spectra of the new chlorins, with bands with high extinction coefficients in the far red region, combined with high singlet oxygen quantum yields for derivatives **4a** and **4c**, suggest their potential efficiency in photodynamic activity.

Experimental Section

General Procedures. Melting points were measured on a Buchi Melting Point B-540 apparatus. 1H and ^{13}C solution NMR spectra were recorded on a Bruker Avance 300 spectrometer at 300.13 and 75.47 MHz, respectively. $CDCl_3$ was used as solvent and TMS as internal reference. Mass spectra were recorded using a MALDI TOF/TOF 4800 Analyzer (Applied Biosystems MDS Sciex) with $CHCl_3$ as solvent and without matrix. The UV-Vis spectra were recorded on a UV-2501 PC Shimadzu spectrophotometer using $CHCl_3$ as solvent. Steady-state fluorescence spectra of the investigated compounds were measured in 1 cm x 1 cm quartz optical cells using a combination of a cw-Xenon lamp (XBO 150) and a monochromator (Lot-Oriel, bandwidth 10 nm) for excitation and a polychromator with a cooled CCD matrix as a detector system (Lot-Oriel, Instaspec IV).²⁷ As reference for measurements of fluorescence quantum yield, Φ_{fl} , the *meso*-tetraphenylporphyrin (TPP) in toluene ($\Phi_{fl} = 0.11$) was used. Photosensitized steady-state singlet oxygen luminescence was measured at 1270 nm. A cw Yb:YAG laser (Versadisk, ELS) equipped with a frequency doubling unit was used to excite the samples at 420 nm. The setup for

detection of the luminescence signal has been reported previously.²⁸ To calculate the singlet oxygen quantum yield, Φ_{Δ} , TPP in toluene was used as reference ($\Phi_{\Delta}=0.68$).

Column chromatography was carried out using silica gel (Merck, 35-70 mesh). Analytical TLC was carried out on precoated sheets with silica gel (Merck, 0.2 mm thick). All the chemicals were used as supplied. Solvents were purified or dried according to the literature procedures.²⁹ The ethyl hydrazono- α -bromoglyoxylates **2a-d** were prepared according to literature procedures.³⁰

Synthesis of the cycloadducts. General procedure

A solution of porphyrin **1** (20 mg, 0.02 mmol) in dry solvent (2 mL), the appropriate ethyl hydrazono- α -bromoglyoxylate (5 equiv.) and base (0.2 mmol), under N₂ atmosphere, was heated in an oil bath for 3 h at the temperature indicated in Table 1. The addition of ethyl hydrazono- α -bromoglyoxylate (5 equiv.) was repeated four times every 3 hours. After the last addition, stirring was continued for 6 h at the same temperature. The reaction mixture was allowed to cool to room temperature, the solvent was evaporated under reduced pressure and the desired chlorin was isolated by column chromatography using a gradient of toluene/ethyl acetate. Chlorins **4a-c** have R_F values in silica lower than porphyrin **1**. The yields are given in Table 1.

4a. mp >300 °C, ¹H NMR (300 MHz, CDCl₃): δ 8.71 (1H, d, J = 4.8 Hz, H- β), 8.65 (1H, d, J = 5.1 Hz, H- β), 8.53-8.41 (3H, m, H- β), 8.26 (1H, d, J = 4.8 Hz, H- β), 7.67 (1H, d, J = 9.1 Hz, H-2), 7.13 (2H, d, J = 8.4, H-Ar), 7.06 (2H, d, J = 8.4, H-Ar), 6.57 (1H, d, J = 9.1 Hz, H-3), 4.05-3.94 (2H, m, CH₂CH₃), 2.41 (3H, s, CH₃), 1.06 (3H, t, J = 7.1 Hz, CH₂CH₃), -1.66 (2H, s, NH) ppm. ¹³C NMR (75 MHz, CDCl₃): δ 162.6, 161.9, 159.9, 153.3, 153.0, 143.1, 141.7, 140.5, 140.1, 135.5, 135.4, 134.6, 132.8, 132.7, 129.5, 128.1, 127.8, 125.1, 125.0, 118.5, 106.7, 106.2, 99.0, 97.7, 77.6 (C-2), 61.5 (CH₂CH₃), 60.5 (C-3), 20.7 (ArCH₃), 13.8 (CH₂CH₃) ppm. UV-Vis (CHCl₃) λ_{\max} (log ϵ) 408 (3.99), 505 (2.93), 535 (2.52), 603 (2.41), 657 (3.25) nm. MS (MALDI): 1179 [M+H]⁺. HRMS-TOF m/z for C₅₅H₂₃F₂₀N₆O₂ [M+H]⁺ calcd 1179.1558, found 1179.1607.

4b. mp >300 °C, ¹H NMR (300 MHz, CDCl₃): δ 8.71 (1H, d, J = 4.8 Hz, H- β), 8.66 (1H, d, J = 4.8 Hz, H- β), 8.49 (2H, s, H- β), 8.42 (1H, d, J = 4.8 Hz, H- β), 8.29 (1H, d, J = 4.8 Hz, H- β), 7.47 (1H, d, J = 9.0 Hz, H-2), 6.98 (2H, d, J = 8.8 Hz, H-Ar), 6.79 (2H, d, J = 8.8 Hz, H-Ar), 6.61 (1H, d, J = 9.0 Hz, H-3), 4.11-4.05 (2H, m, CH₂CH₃), 3.85 (3H, s, OCH₃), 0.89 (3H, t, J = 8.3 Hz, CH₂CH₃), -1.66 (2H, s, NH). ¹³C NMR (75 MHz, CDCl₃): δ 162.0, 161.4, 160.8, 157.7, 153.2, 142.4, 140.4, 140.1, 137.6, 135.53, 135.45, 132.8, 128.0, 127.9, 125.2, 124.9, 122.6, 114.2, 106.53, 106.47, 99.6, 98.7, 79.9 (C-2), 61.5 (CH₂CH₃), 60.8 (C-3), 55.6 (OCH₃), 13.9 (CH₂CH₃) ppm. UV-Vis (CHCl₃) λ_{\max} (log ϵ) 408 (5.11), 506 (4.06), 534 (3.68), 603 (3.54), 654 (4.35) nm. MS (MALDI): 1195 [M+H]⁺. HRMS-TOF m/z for C₅₅H₂₃F₂₀N₆O₃ [M+H]⁺ calcd 1195.1507, found 1195.1522.

4c. mp >300 °C, ¹H NMR (300 MHz, CDCl₃): δ 8.70 (1H, d, J = 4.9 Hz, H- β), 8.65 (1H, d, J = 5.0 Hz, H- β), 8.50-8.44 (3H, m, H- β), 8.24 (1H, d, J = 5.0 Hz, H- β), 7.66 (1H, d, J = 8.9 Hz, H-2), 7.31 (2H, d, J = 8.5 Hz, H-Ar), 7.14 (2H, d, J = 8.5 Hz, H-Ar), 6.52 (1H, d, J = 8.9 Hz,

H-3), 3.99-3.91 (2H, m, CH₂CH₃), 1.01 (3H, t, *J* = 7.1 Hz, CH₂CH₃), -1.69 to -1.66 (2H, m, NH) ppm. UV-Vis (CHCl₃) λ_{max} (log ε) 408 (5.11), 509 (4.06), 535 (3.68), 602 (3.54), 658 (4.35) nm. MS (MALDI): 1199 [M+H]⁺. HRMS-TOF *m/z* for C₅₄H₂₀ClF₂₀N₆O₂ [M+H]⁺ calcd 1199.1011, found 1199.1058.

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