Novel cycloimides in the chlorophyll *a* series

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The reaction of hydrazine hydrate with purpurin 18 was studied. The resulting monohydrazide readily reacts with the second carboxy group on acidification to give a six-membered chlorin p_6 N-aminocycloimide with the reduction of the vinyl group to ethyl.

Over the last years, natural chlorins have been attracting much interest as possible photosensitisers (PS) for the photodynamic therapy of cancer.[1] The reason for this is that these compounds have high quantum yields for singlet oxygen and absorption in the range 650–700 nm, where biological tissues possess enhanced transparency.[2]

The creation of high-efficiency chlorin-based PS is a complex problem, involving the development of methods to synthesise stable chlorophyll *a* derivatives with improved spectral and photophysical characteristics, the construction of novel amphiphilic molecules with optimal hydrophobic-to-hydrophilic substituent ratios, and increasing the efficiency and selectivity of the photodynamic effect of sensitisers.

A promising way to enhance the stability of chlorophyll *a* derivatives is their transformation to cyclic chlorin p_6 imides.[3–5] Ionised groups or hydroxyl-containing substituents can be introduced in the PS molecules to increase their hydrophilicity and solubility in water by modifying the vinyl substituent in pyrrole A[6–8] or the propionic acid residue.[9,10] The development of a method to obtain N-hydroxycycloimides in the chlorin[11] or bacteriochlorin series[12] opened new prospects for the synthesis of PS containing various hydrophilic and hydrophobic substituents in the exo-cycle.

Obviously, the applicability of the methods of organic chemistry for solving the above problems might be expanded considerably if the pigment molecule contained a reactive amino group.[13] In this work, we developed a convenient method to introduce such a substituent into chlorins by the reaction of hydrazine hydrate with the anhydride ring of purpurin 18.

The transformation of the anhydride to a cyclic imide involves two main steps. In the first step, the opening of the anhydride ring occurs, accompanied by a hypsochromic shift of band Q to the region around 670 nm, to give monohydrazide **2**. On treatment with hydrochloric acid, compound **2** undergoes intramolecular cyclisation to give N-aminocycloimide **3** (Scheme 1). The structure of its methyl ester **4** was confirmed by the ^{1}H NMR† spectrum, which showed a distinct signal of two protons of the exocyclic amino group as a slightly broadened singlet at δ 5.7 ppm. The amino group readily undergoes alkylation or tosylation. In fact, the treatment of cycloimide **4** with methyl iodide or tosyl chloride gave N,N-dimethylamino derivative **5** or N-tosyl derivative **6**, respectively.

It was shown for *p*-nitrobenzaldehyde that it is possible to use the primary amino group in cycloimide **4** to add biomolecules containing a formyl group; the structure of the resulting Schiff base was proven by mass spectra (M$^+$ 727).

Unexpectedly, the reaction of purpurin 18 with hydrazine involved the reduction of the vinyl group at the macrocycle.

It is well known that the use of hydrazine as a reducing agent requires that a base is present, like in certain other cases: the Wolff–Kishner reduction of carbonyl compounds, the use of disperse metals as catalysts or the formation of diimines in the presence of oxidants.[14]

In our system, the reduction of the vinyl group in purpurin 18 was observed irrespective of the reaction medium (pyridine or a chloroform–methanol mixture), and it was a time-dependent process. The reduction dynamics was monitored using ^{1}H NMR (Figure 1) and electronic spectroscopy. However, the chromatographic mobilities of 3-vinyl and 3-ethyl cycloimide derivatives did not differ noticeably. The reaction gave a 1:1 mixture of the above derivatives after 24 h [Figure 1(*b*)], whereas chlorin p_6 3-ethyl-3-devinyl-*N*-aminocycloimide **3** became the major product after 48 h [Figure 1(*c*)]. The spectrum of the latter contains additional signals, namely a quartet (δ 3.78 ppm) and a triplet (δ 1.67 ppm) corresponding to the newly formed ethyl group. The reduction was accompanied by a hypsochromic shift of the main absorption band from 715 to 703 nm, also suggesting that the electronic system of the molecule has changed.

Reduction with hydrazine hydrate, as described above, provides another way to introduce an ethyl group into di- and tetrahydroporphyrin macrocycles.

A preliminary *in vitro*‡ assay of the phototoxicity of cycloimide **4** and its N,N-dimethyl derivative **5** showed that they possess high photoinduced activity and no dark toxicity.

Thus, the synthesised hydrazides of the chlorophyll *a* series are promising second-generation PS. The presence of an amino

† The electronic spectra were recorded in chloroform using a Jasco-UV 7800 spectrophotometer. The ^{1}H NMR spectra were measured in $CDCl_3$ on Bruker WM 250, Bruker WM 300 and DRX 500 spectrometers using the DISNMR94 program. The mass spectra were obtained on a VISION 2000 time-of-flight mass spectrometer using the MALDI method, with dihydroxybenzene (DHB) as the support. The high-resolution mass spectra (HRMS) were recorded on a Micromass Autospec mass spectrometer (electron ionisation energy, 70 eV; 200 °C). The IR spectra were measured in KBr pellets on a Nicolet Magna 750 spectrometer. Column chromatography was carried out on L 40/100 silica gel (Chemapol). Preparative TLC was performed using Silica Gel 60 (Merck) on 20×20 cm plates; the layer thickness was 1 mm. Analytical TLC was carried out on Kieselgel 60 F_{245} plates (Merck).

The compounds obtained had the following characteristics:

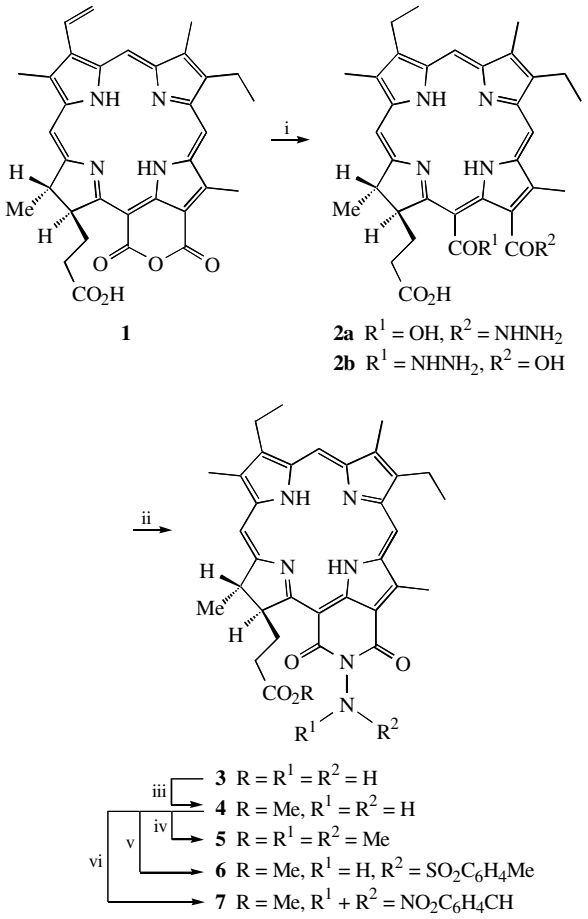
4: ^{1}H NMR, δ: 9.48 (s, 10-H), 9.1 (s, 5-H), 8.42 (s, 20-H), 5.7 (s, NH_2), 5.28 (d, 17-H, *J* 8 Hz), 4.32 (m, 18-H), 3.72 (m, 12-Me and 3^1-CH_2), 3.6 (m, 17^5-Me and 8^1-CH_2), 3.25 (s, 2-Me), 3.18 (s, 7-Me), 2.78 (m, 17^1-CH_2), 2.48 (m, 17^2-CH_2), 1.72 (d, 18-Me, *J* 8 Hz), 1.55 (d, 18-Me, *J* 8 Hz), 0.4 (s, NH), 0.22 (s, NH). UV-VIS, λ_{max}/nm (relative intensities): 366, 420, 549.6, 703.2 (0.55:1:0.19:0.33). R_f (5:2 v/v chloroform–methanol) 0.5. IR, ν/cm^{-1}: 1734, 1687, 1642, 1600, 1526. MS, *m*/*z* (%): 594.3 (M$^+$, 100). HRMS: calc. for $C_{34}H_{38}N_6O_4$, 594.2954; found, 594.2948.

5: ^{1}H NMR, δ: 9.62 (s, 10-H), 9.2 (s, 5-H), 8.52 (s, 20-H), 5.3 (d, 17-H, *J* 8 Hz), 4.35 (m, 18-H), 3.84 (s, 12-Me), 3.78 (q, 3^1-CH_2, *J* 8 Hz), 3.68 (q, 8^1-CH_2, *J* 8 Hz), 3.58 (s, 17^5-Me), 3.4 (s, NMe_2), 3.26 (s, 2-Me), 3.21 (s, 7-Me), 2.82 (m, 17^1-CH_2), 2.45 (m, 17^2-CH_2), 1.7 (d, 18-Me, *J* 8 Hz), 1.67 (m, 3^2-Me, 8^2-Me), 0.05 (s, NH), –0.15 (s, NH). UV-VIS, λ_{max}/nm (relative intensities): 363.2, 416.6, 544, 694.6 (0.37:1:0.15:0.32). R_f (chloroform) 0.4. IR, ν/cm^{-1}: 1734, 1687, 1641, 1600, 1526. HRMS: calc. for $C_{36}H_{42}N_6O_4$, 622.3271; found, 622.3274.

6: ^{1}H NMR, δ: 9.52 (s, 10-H), 9.14 (s, 5-H), 8.44 (s, 20-H), 8.01 (d, CH-arom., *J* 8 Hz), 8.0 (s, NH), 7.31 (d, CH-arom., *J* 8 Hz), 5.0 (d, 17-H, *J* 8 Hz), 4.29 (m, 18-H), 3.73 (m, 12-Me and 3^1-CH_2), 3.6 (m, 17^5-Me and 8^1-CH_2), 3.22 (s, 2-Me), 3.15 (s, 7-Me), 2.63 (m, 17^1-CH_2), 2.44 (s, Me-arom.), 2.38 (m, 17^2-CH_2), 1.7 (d, 18-Me, *J* 8 Hz), 1.66 (m, 3^2-Me, 8^2-Me), 0.4 (s, NH), 0.20 (s, NH). UV-VIS, λ_{max}/nm (relative intensities): 363.0, 416.0, 544.5, 695.5 (0.36:1.0:0.15:0.3). MS, *m*/*z* (%): 749.5 (M$^+$, 100).

7: MS, *m*/*z* (%): 727.4 (M$^+$, 100)

‡ The bioassay was carried out at the P. A. Hertsen Moscow Research Oncological Institute under the supervision of Professor R. I. Yakubovskaya.



Scheme 1 Synthesis of chlorin p_6 N-aminocycloimide and its chemical modifications. *Reagents and conditions*: i, N_2H_4, 48 h; ii, 1 N HCl, 2 h; iii, CH_2N_2, Et_2O; iv, MeI; v, TosCl, Py; vi, $NO_2C_6H_4CHO$, C_6H_6, reflux.

group in the molecule opens up additional prospects to functionalise the chlorin macrocycle, including the creation of cationic PS and conjugates with various biomolecules in order to improve their directed transportation to the tumour and to increase tumour selectivity.

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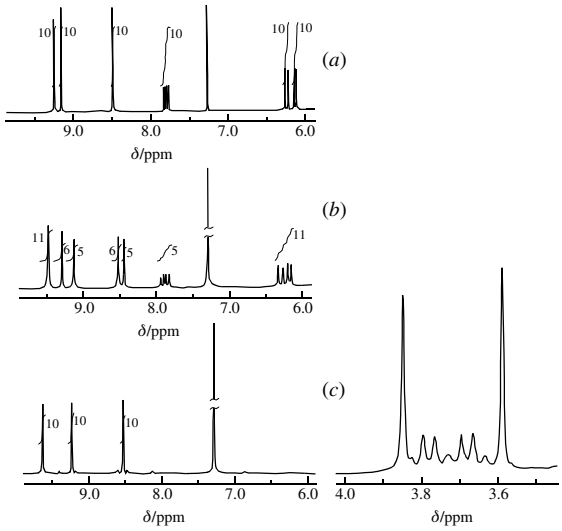

Figure 1 Fragments of the ^{1}H NMR spectra of substituted cycloimides **3**: (*a*) 3-vinyl-**3**, (*b*) a mixture of 3-vinyl- and 3-ethyl-**3** and (*c*) 3-ethyl-**3**.

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