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Mendeleev Commun., 2004, 14(5), 204–207

Mendeleev Communications

Synthesis of cationic bacteriochlorins

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DOI: 10.1070/MC2004v014n05ABEH001941

A group of positively charged bacteriochlorins has been obtained for the first time from natural bacteriochlorophyll *a* (*Bchl a*).

The development of efficient means for fighting microbial contamination is among the most important tasks of microbiology and medicine. This problem has recently become particularly pressing due to the increasing acquired resistance of bacterial and fungal pathogens to chemotherapy (antibiotics, antimycotics).[1,2]

Photodynamic antimicrobial chemotherapy, which is based on the inactivation of viruses, bacteria, yeast fungi and protozoa by the active forms of oxygen generated by photosensitizers in an excited state,[3] is currently a subject of active development.

An analysis of available literature data shows that the above method is far behind the antineoplastic photodynamic therapy as regards the level of its fundamental development and practical implementation. Only isolated data are available on the photosensitization of nonpathogenic yeasts in the presence of porphyrins and phthalocyanins and *Candida albicans* pathogenic yeast fungi in the presence of phenothiazines and hematoporphyrin.[4–9]

It is well known that the external surfaces of bacterial cells bear a negative charge; as a result, the most efficient binding with bacterial cells, and hence the photodynamic effect, should be expected in the case of cationic photosensitizers.

The aim of this work was to synthesise and analyse the antimicrobial activity of cationic cyclic imides in the bacteriochlorophyll *a* series.

We decided to use bacteriochlorin *p* *N*-aminocycloimide **1** and its *N*,*N*-dimethylamino derivative[10] as starting compounds. However, attempts to perform the quaternization of the nitrogen atom in the latter compound using methyl iodide or dimethyl sulfate failed, probably, owing to deactivation of the amino group with the neighbouring cycloimide.

A different approach, which involved the introduction of the isonicotinyl residue into the amino group followed by quaternization at the pyridine ring (Scheme 1), was more successful. This was carried out by the treatment of cycloimide **1** with isonicotinyl chloride in pyridine. The chromatographic mobility of the acylation product was much lower than that of the starting cycloimide. The structure of compound **2** was confirmed by mass spectrometry, the most intense peak with *m*/*z* 716 was due to the molecular ion.

The structure of hydrazide **2** was also supported by the ^{1}H NMR spectrum,† though the signals of all protons were doubled, as one can see in Figure 1 (the region of *meso*-H and pyridine ring protons is shown). This fact can be explained by

the existence of isomers with very similar chromatographic mobilities, which excludes the possibility of their preparative separation. The existence of isomeric forms of hydrazides is probably due to both keto–enol tautomerism, which gives iminol **4**, and to possible stereoisomerism owing to the absence of free rotation about the C(O)–N bond (Scheme 2).

The availability of two nucleophilic atoms (O and N) in isomers **2** and **4** made it possible to methylate them with diazomethane, which resulted in isomers **5** and **6** with the same molecular mass but different chromatographic mobilities.

Pure compounds **5** and **6** were isolated using preparative TLC on silica gel. The structure of **5** with a methyl group at the nitrogen atom was assigned to the more mobile isomer. The structure of **6** with a methyl group at oxygen was assigned to the second isomer. The structure of the former isomer was proved by independent synthesis (Scheme 3). For this purpose, *N*-aminocycloimide **1** was treated with methyl iodide in the 1:1

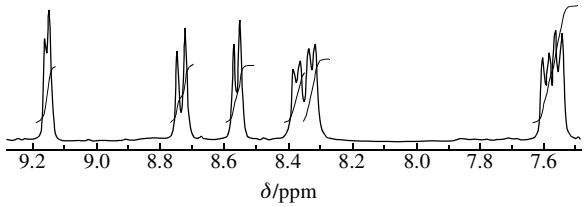


Figure 1 Fragment of the ^{1}H NMR spectrum of cycloimide **2**.

† The electronic absorption spectra were recorded in $CHCl_3$ with a Jasco-UV 7800 spectrophotometer. The ^{1}H NMR spectra were obtained in $CDCl_3$ using Bruker WM 250, WM 300 and DRX 500 instruments and the DISNMR94 software. The mass spectra were measured using a VISION 2000 time-of-flight MS instrument by MALDI with 2,5-dihydroxybenzoic acid as a matrix.

The compounds obtained had the following characteristics.

2: ^{1}H NMR ($CDCl_3$, 333 K) δ: 9.15 (s, 10-H), 8.95 (d, 2H of pyridine, *J* 8 Hz), 8.78 (s, 5-H), 8.56 (s, 20-H), 8.2 (d, 2H of pyridine, *J* 8 Hz), 5.20 (m, 17-H), 4.30 (m, 18-H, 7-H), 4.1 (m, 8-H), 3.68 (s, 12-Me), 3.55 (s, 17^5-Me), 3.50 (s, 2-Me), 3.18 (s, 3^2-Me), 2.75 (m, 8^1-CH_2), 2.4 (m, 17^2-CH_2), 2.1 (m, 17^1-CH_2), 1.85 (d, 7-Me, *J* 7 Hz), 1.70 (d, 18-Me, *J* 8 Hz), 1.12 (t, 8^2-Me, *J* 7 Hz), –0.25 (s, NH), –0.5 (s, NH). UV-VIS, λ_{max}/nm (ε/10^{-3} dm^3 mol^{-1} cm^{-1}): 369.5 (57), 422.5 (30.4), 555.5 (27.4), 834.5 (35.4). MS, *m*/*z* (%): 716 (M^+, 100).

3: ^{1}H NMR (CD_3OD/D_2O) δ: 9.2 (m, 10-H, 2H of pyridine), 8.82 (s, 5-H), 8.72 (d, 2H of pyridine, *J* 8 Hz), 8.65 (s, 20-H), 5.15 (m, 17-H), 4.30 (m, 18-H, 7-H), 4.1 (m, 8-H), 4.0 (s, N–Me), 3.70 (s, 12-Me), 3.55 (s, 17^5-Me), 3.53 (s, 2-Me), 3.18 (s, 3^2-Me), 2.70 (m, 8^1-CH_2), 2.4 (m, 17^2-CH_2), 2.05 (m, 17^1-CH_2), 1.80 (d, 7-Me, *J* 7 Hz), 1.70 (d, 18-Me, *J* 8 Hz), 1.10 (t, 8^2-Me, *J* 7 Hz), –0.3 (s, NH), –0.5 (s, NH). UV-VIS, λ_{max}/nm (ε/10^{-3} dm^3 mol^{-1} cm^{-1}): 366.0 (53.5), 417.0 (29.0), 550.0 (22.5), 830.5 (35.7). MS, *m*/*z* (%): 731 (M^+, 100).

5: MS, *m*/*z* (%): 730.5 (M^+, 100).

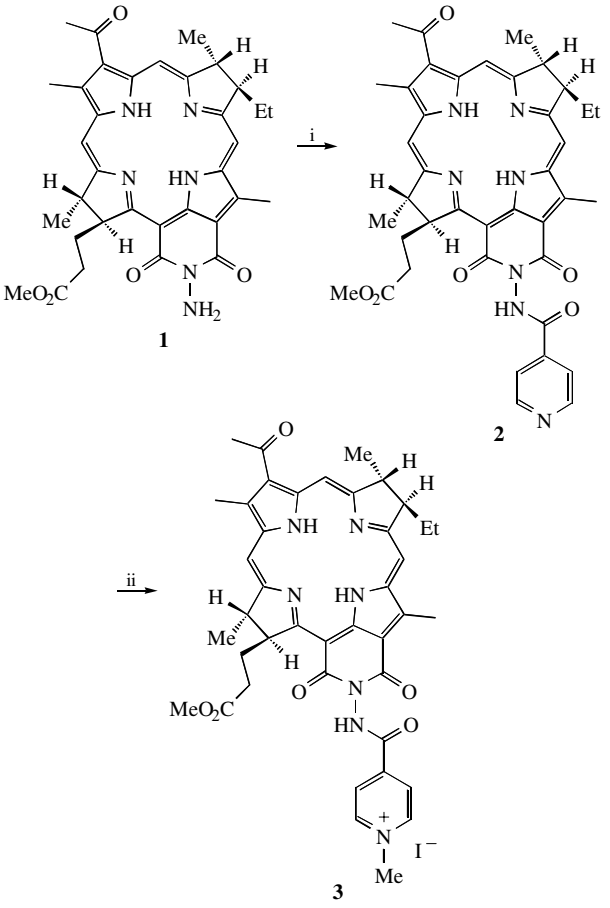
6: ^{1}H NMR ($CDCl_3$) δ: 9.21 (s, 10-H), 8.87 (d, 2H of pyridine, *J* 8 Hz), 8.80 (s, 5-H), 8.62 (s, 20-H), 7.98 (d, 2H of pyridine, *J* 8 Hz), 5.3 (m, 17-H), 4.30 (m, 18-H, 7-H), 4.1 (m, 8-H), 4.0 (s, O–Me), 3.70 (s, 12-Me), 3.55 (s, 17^5-Me), 3.53 (s, 2-Me), 3.18 (s, 3^2-Me), 2.70 (m, 8^1-CH_2), 2.4 (m, 17^2-CH_2), 2.05 (m, 17^1-CH_2), 1.80 (d, 7-Me, *J* 7 Hz), 1.70 (d, 18-Me, *J* 8 Hz), 1.10 (t, 8^2-Me, *J* 7 Hz), –0.3 (s, NH), –0.5 (s, NH). MS, *m*/*z* (%): 730.5 (M^+, 100), 611.4 ($M^+ - C_5H_4NCOMe$, 17).

7: MS, *m*/*z* (%): 623.2 (M^+, 100), 593.2 (M^+ – NHMe).

8: ^{1}H NMR ($CDCl_3$) δ: 10.31 (s, NH-amide), 9.22 (s, 10-H), 8.82 (s, 5-H), 8.61 (s, 20-H), 8.18 [d, 3(5)-H of pyridine, *J* 8 Hz], 7.82 (dd, 4-H of pyridine, *J* 8 Hz), 7.45 [d, 5(3)-H of pyridine, *J* 8 Hz], 5.28 (m, 17-H), 4.33 (m, 18-H, 7-H), 4.14 (m, 8-H), 3.74 (s, 12-Me), 3.58 (s, 17^5-Me, 2-Me), 3.23 (s, 3^2-Me), 2.75 (m, Me of pyridine ring, 8^1-CH_2), 2.14 (m, 17^2-CH_2), 2.02 (m, 17^1-CH_2), 1.88 (d, 7-Me, *J* 7 Hz), 1.72 (d, 18-Me, *J* 8 Hz), 1.17 (t, 8^2-Me, *J* 7 Hz), –0.25 (s, NH), –0.52 (s, NH). UV-VIS, λ_{max}/nm: 365.0, 416.0, 548.5, 829.0. MS, *m*/*z* (%): 730.3 (M^+, 100).

9: ^{1}H NMR ($CDCl_3$, 333 K) δ: 9.58 (m, 4-H in pyridine ring), 9.45 (NH-amide), 9.18 (s, 10-H), 8.76 (s, 5-H), 8.65 (d, 6-H of pyridine, *J* 2 Hz), 8.6 (s, 20-H), 7.55 (d, 3-H of pyridine, *J* 8 Hz), 5.20 (m, 17-H), 4.28 (m, 18-H, 7-H), 4.10 (m, 8-H), 3.68 (s, 12-Me), 3.52 (s, 17^5-Me, 2-Me), 3.15 (s, 3^2-Me), 2.85 (s, Me of pyridine), 2.65 (8^1-CH_2), 2.40 (m, 17^2-CH_2), 2.05 (m, 17^1-CH_2), 1.85 (d, 7-Me, *J* 7 Hz), 1.70 (d, 18-Me, *J* 8 Hz), 1.12 (t, 8^2-Me, *J* 7 Hz), –0.25 (s, NH), –0.52 (s, NH). UV-VIS, λ_{max}/nm: 366, 417.0, 549.5, 829.5. MS, *m*/*z* (%): 730.2 (M^+, 100).

10: MS, *m*/*z* (%): 745 (M^+, 100).



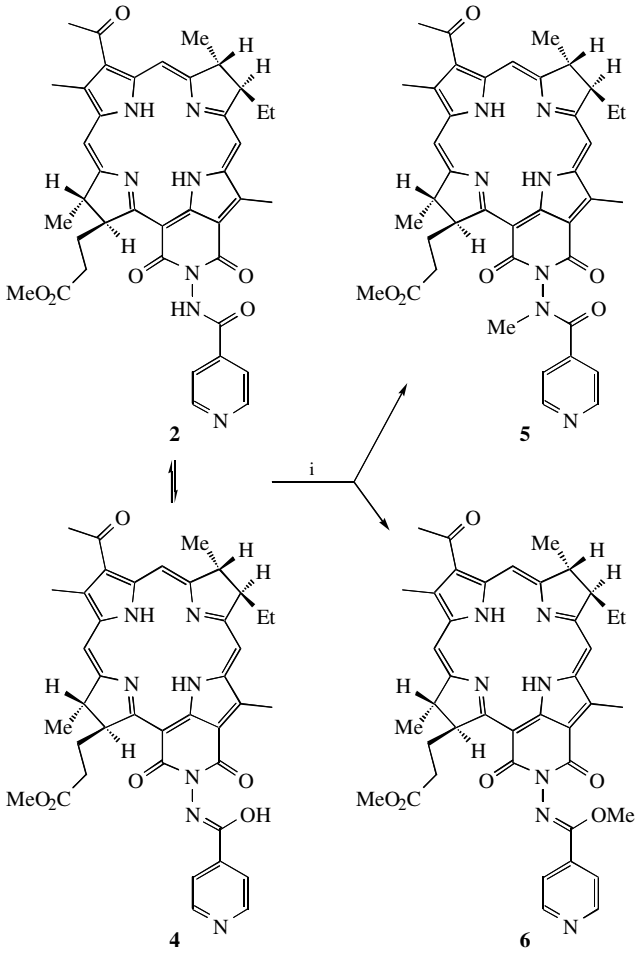
Scheme 1 Synthesis of a cationic cycloimide with an isonicotinic fragment. *Reagents and conditions*: i, C_5H_4NCOCl, Py, 15 min; ii, MeI, reflux.

ratio; the resulting *N*-methylaminocycloimide **7** was separated by TLC from starting compound **1** and the *N*,*N*-dimethylamino-substituted compound. The structure of compound **7** was confirmed by mass spectrometry (M+, 623.2). The subsequent acylation with isonicotinyl chloride gave compound **5**, the physico-chemical characteristics of which totally matched those of the product obtained upon the treatment of compound **2** with diazomethane (Scheme 2).

The second isomer (compound **6**) was found to be less stable; it underwent rapid decomposition to give *N*-aminocycloimide **1**, which was detected using TLC. The mass spectrum of *O*-methylimidate **6** contains, in addition to the molecular ion with *m*/*z* 730, a peak with *m*/*z* 610, which corresponds to cycloimide **1**. The latter was treated with methyl iodide; the molecular mass and chromatographic mobility of the resulting compound was identical to those of *N*,*N*-dimethylaminocycloimide of bacteriochlorin *p*.[11]

The ^{1}H NMR spectra of isomer **5** showed signal doubling, which was noted above for starting hydrazide **2**. This phenomenon apparently results from the existence of another type of isomerism in amides, which appears due to the absence of free rotation about the C(O)–N bond. Since the rotation barrier is not high for such isomers, it can be easily overcome by increasing temperature. In fact, the ^{1}H NMR signals of compounds **2** and **5** merged on heating to 50 °C. On the other hand, such a doubling was not observed in the ^{1}H NMR spectra of isomer **6** recorded during the first hours after isolating the product; this indicates the presence of a single isomer, the possible structure of which is shown in Scheme 2.

Quaternization of hydrazide **2** was carried out by treatment with methyl iodide. The resulting compound **3** had an extremely low chromatographic mobility, which confirmed the existence of a charge in the molecule. The mass spectrum of the resulting cycloimide and its ^{1}H NMR spectra, which contain an additional singlet of the methyl group at the nitrogen atom of the hetero-

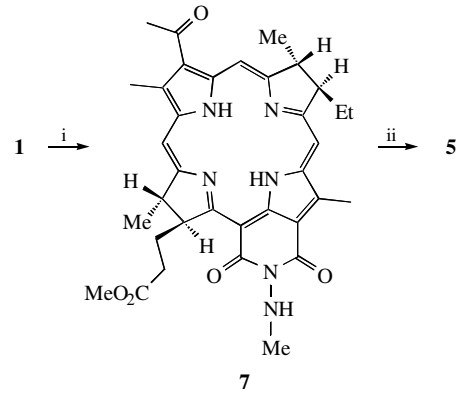


Scheme 2 Reactions of cycloimides **2** and **4** with diazomethane. *Reagents and conditions*: i, CH_2N_2, Et_2O, room temperature, 15 min.

cycle, decisively confirmed the structure of the quaternary pyridinium salt.

In order to estimate the effect of structural features of heterocyclic fragments introduced in photosensitizer (PS) molecules on the chemical activity and isomerism of the latter, we used 6-methylpicolinyl chloride and 6-methylnicotinyl chloride as acylating agents (Scheme 4). We found that the ^{1}H NMR spectra of hydrazide **8**† containing the 6-methylpicolinyl residue did not show the doubling of protons, presumably owing to the absence of rotation of the pyridine ring because of the formation of an intramolecular hydrogen bond between the nitrogen atom of the heterocycle and the amide hydrogen (Figure 2).

In this case, a five-membered ring is formed, which results in the rigid fixation of the pyridine ring in the plane of the amide bond. Attempts at the quaternization of the nitrogen atom in hydrazide **8** also failed, presumably, due to deactivation of the nitrogen atom of the heterocycle by the adjacent carbonyl group.



Scheme 3 Synthesis of *N*-methyl-*N*-isonicotinylhydrazide **5**. *Reagents and conditions*: i, MeI, 2 h, reflux; ii, C_5H_4NCOCl, Py, 15 min.

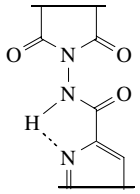


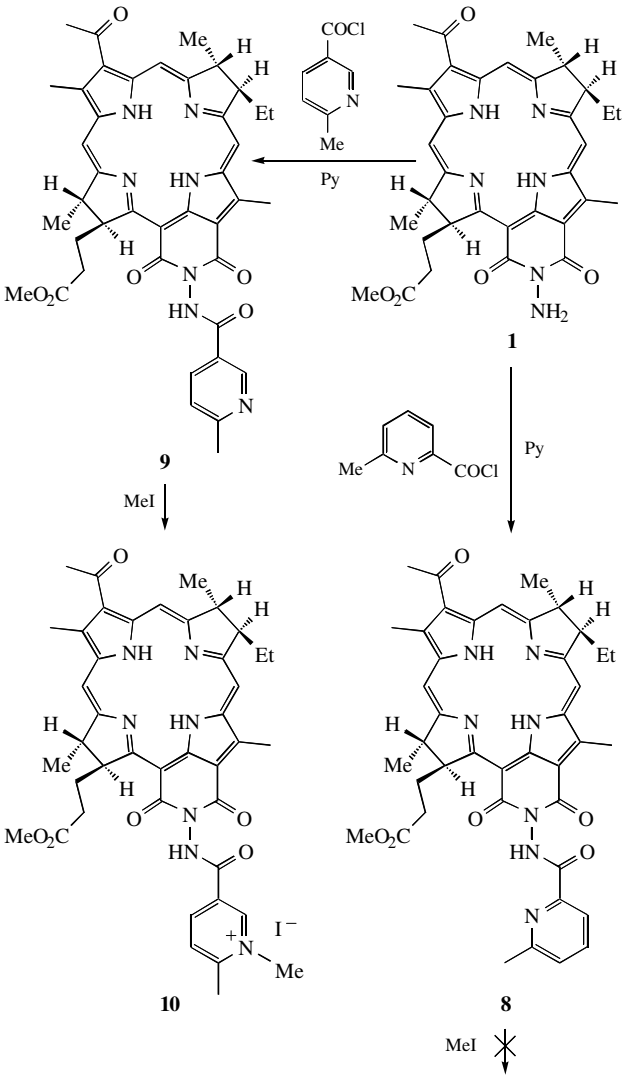
Figure 2 Fragment of the structure of cycloimide **8** with a 6-methylpicolinyl residue.

An increase in the distance between the nitrogen atom and the carboxyl group in the 6-methylnicotinyl residue facilitates the acylation of cycloimide **1** followed by the quaternization of hydrazide **9**.†

Cationic PSs of bacteriochlorin type are soluble in water–alcohol solutions, which made it possible to prepare water-soluble pigment forms for biological tests without the use of Cremophor EL (a derivative of castor oil and ethylene oxide).[12,13]

Taking into account the long-wave absorption maximum of the cationic cycloimide at 830 nm (the region of the high optical transparency of biological media), the latter compound combined with a suitable radiation source can be used for the photosensitization of dense microorganism cultures.

This study was supported by the Russian Foundation for Basic Research (grant nos. 01-03-032543 and 00-15-97866), INTAS (grant no. 01-0461) and a joint grant of the Russian Foundation for Basic Research (grant no. 02-04-08043) and the Foundation for Assistance to Small Innovative Enterprises.



Scheme 4 Synthesis and quaternization of cycloimides containing pyridinecarboxylic acid residues.

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Received: 29th March 2004; Com. 04/2266