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The synthesis and characterization of metal-free, unsymmetrical azaphthalocyanines with hydroxy groups and their complex formation with pyridine

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Abstract

Three, unsymmetrical metal-free azaphthalocyanines (AzaPc) were prepared using the statistical condensation of 5,6-bis(diethylamino)-pyrazine-2,3-dicarbonitrile (A) and the pyrazinedicarbonitrile (B) substituted with alkylamine chains bearing one or two hydroxy groups. The desired AAAB type, metal-free compounds were isolated, purified and characterized; the compounds contain one or two hydroxy groups that can be modified with suitable ligands. The mechanism leading to the unusual formation of a new morpholine ring during the preparation of some pyrazinedicarbonitriles is explained. Metal-free AzaPcs form a proton-transfer complex with two molecules of pyridine, this complex formation being accompanied by a change of solution color from purple to blue. The complex is formed directly with two molecules of pyridine, one on either side of the macrocycle. The rate constants of this process were found to be of the order $\sim 10^{-4}$ s^{-1}; the rate of complex formation was not the same for all compounds and may depend on the AzaPc structure. The influence of hydroxy groups on the rate constant was not confirmed.

Keywords: Azaphthalocyanine; Tetrapyrazinoporphyrazine; Statistical condensation; Pyridine complex; UV–vis spectroscopy

1. Introduction

Phthalocyanines (Pc) and their derivatives (e.g. azaanalogues – azaphthalocyanines, AzaPc) are interesting macrocyclic compounds used nowadays in different applications – commercial dyes and pigments [1], chemical sensors [2], liquid crystals [3], photodynamic [4] or photothermal [5] therapy. Symmetrical Pc and AzaPc are simple to prepare by tetramerization of one precursor. However, unsymmetrical Pc or AzaPc with one or two different substituents on periphery are more advantageous in some cases, e.g. as building blocks of Pc polymers [6], for the third generation photosensitizers [7] or for binding to different surfaces [8]. In such a case, synthesis is more complicated. Although some selective methods were developed for synthesis of Pc with one different unit [9–11], the most widespread method is still a statistical condensation of two differently substituted precursors (A and B). This reaction leads to appearance of a mixture of all possible derivatives (AAAA, AAAB, AABB, ABAB, ABBB and BBBB) that must be chromatographically separated. The isolation of the products of statistical condensation is often complicated by strong tendency of planar Pc molecules to aggregation. Introduction of bulky substituents on periphery of Pc inhibits efficiently the aggregation [12,13] and subsequently enables successful chromatographic isolation of the products [14,15]. Theoretical statistical calculations say that the ratio 3:1 of precursors A/B gives the highest yields of AAAB type under conditions that reactivity of both precursors is similar and there is no sterical constraint between adjacent isoindol subunits of Pc. The method of statistical condensation is suitable not only for Pc but also for dyes of mixed character,

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for example compounds that originate from reaction of phthalonitriles with substituted maleonitriles [16,17].

Metal phthalocyanines (MPc) are known to form the complexes with pyridine through coordination of the central metal [8]. Such complexes increase their solubility in organic solvents where the MPc alone are not soluble (e.g. ethanol). That is why, some procedures (e.g. liposome preparation [18,19]) take advantage of such pyridine complex formation. However, metal-free Pc were also shown to form complexes with pyridine. The central hydrogens can be almost removed to form so-called ''proton-transfer complex'' with molecules of weak bases (e.g. pyridine and amines) [20]. Recently, we have described such behavior also for AzaPc of tetrapyrazinoporphyrazine type [21].

The aim of this work was to prepare unsymmetrical AzaPc with one or two hydroxy groups for future binding of peripheral ligands. We also investigated the formation of complexes of such metal-free AzaPc with pyridine and a kinetic of the process.

2. Experimental

All organic solvents used for the synthesis were of analytical grade. Anhydrous butanol was stored over magnesium and distilled prior to use. Pyridine for complex formation was from Lach-Ner, Czech Republic. All chemicals were used as received. TLC was performed on Merck aluminium sheets with silica gel 60 F254. Merck Kieselgel 60 (0.040–0.063 mm) was used for column chromatography. Melting points were measured on Electrothermal IA9200 Series Digital Melting Point Apparatus (Electrothermal Engineering Ltd., Southend-on-Sea, Essex, Great Britain) and are uncorrected. Infrared spectra were measured in KBr pellets on IR-Spectrometer Nicolet Impact 400. ^{1}H and ^{13}C NMR spectra were recorded on Varian Mercury − Vx BB 300 (299.95 MHz − ^{1}H and 75.43 MHz − ^{13}C). Chemical shifts reported are given relative to internal $Si(CH_3)_4$. The elemental analyses were carried out on Automatic Microanalyser EA1110CE (Fisons Instruments S.p.A., Milano, Italy). UV–vis spectra were recorded on spectrophotometer UV-2401PC, Shimadzu Europa GmbH (Duisburg, Germany). ESI mass spectra were measured on Quattro Micro™ API (Waters, Milford, Massachusetts, USA) in positive electrospray mode. Solutions for ESI MS were prepared in acetonitrile (for compound **12**) and in methanol (for the rest of the dyes), and formic acid was added before measurements to support ionization. Adducts with Na^+, K^+ and formic acid were always found in the mass spectra of the dyes as minor peaks.

2.1. Synthesis

Compounds **1** [22] and **10** [23] were prepared according to published procedures in a good purity. Compound **12** that was isolated from the reaction mixtures showed characteristics (R_f values, UV–vis, NMR, IR spectra) identical with those of the same compound prepared before in our laboratory [21] by simple tetramerization of **10**, and therefore it is not characterized further.

2.1.1. 5-Chloro-6-diethylaminopyrazine-2,3-dicarbonitrile (2)

A solution of **1** (2.4 g, 12 mmol) in tetrahydrofuran (100 mL) was cooled to −12 °C using ice/salt bath and diethylamine (1.77 g, 24 mmol) was added dropwise. The reaction mixture was left to warm slowly to r.t. and precipitated diethylamine hydrochloride was filtered off. The rest was evaporated under reduced pressure and purified by column chromatography on silica with hexane/ethyl acetate 4:1 as eluent. Yield 2.66 g (93%) of yellow oily liquid; IR, ν (cm^{-1}): 2987, 2939, 2876, 2233 (CN), 1553, 1507, 1439, 1386 and 1358; ^{1}H NMR ($CDCl_3$) δ: 3.75 (4H, q, $J = 7.1$ Hz, CH_2) and 1.31 (6H, t, $J = 7.0$ Hz, CH_3); ^{13}C NMR ($CDCl_3$) δ: 151.3, 135.0, 139.4, 117.3, 113.2, 113.0, 45.5 and 13.0.

2.1.2. 5-Diethylamino-6-[2-(2-hydroxyethoxy)-ethylamino]-pyrazine-2,3-dicarbonitrile (3)

A solution of **2** (1.0 g, 4.25 mmol) in tetrahydrofuran (50 mL) was stirred at r.t. and 2-(2-aminoethoxy)ethanol (1.34 g, 12.75 mmol) was added dropwise. Reaction continued at r.t. for next 5 h. The mixture was then evaporated under reduced pressure, dissolved in chloroform and extracted three times with water. The organic layer was dried over Na_2SO_4, evaporated and purified by column chromatography on silica with dichloromethane/acetone 3:1 as eluent. Yield 996 mg (77%) of yellowish oily liquid; IR, ν (cm^{-1}): 3424, 2974, 2935, 2875, 2231 (CN), 1560, 1537, 1504, 1457, 1436, 1358 and 1265; ^{1}H NMR (acetone-d_6) δ: 6.76–6.54 (1H, br s, NH), 3.73 (2H, t, J 5.5 Hz, CH_2–O), 3.70–3.61 (4H, m, CH_2–O), 3.61–3.53 (2H, m, CH_2–NH), 3.45 (4H, q, $J = 7.1$ Hz, CH_2–N) and 1.15 (6H, t, $J = 7.1$ Hz, CH_3); ^{13}C NMR (acetone-d_6) δ: 148.6, 148.4, 123.8, 118.8, 116.2, 116.0, 73.2, 69.0, 62.0, 44.1, 41.9 and 12.9.

2.1.3. 5,6-Bis[2-(2-hydroxyethoxy)-ethylamino]-pyrazine-2,3-dicarbonitrile (4)

A solution of **1** (500 mg, 2.5 mmol) in tetrahydrofuran (50 mL) was stirred at r.t. and 2-(2-aminoethoxy)ethanol (1.12 g, 10.7 mmol) was added dropwise. Reaction continued at r.t. for next 3 h. The solution was then decanted from oily drops, evaporated under reduced pressure and purified by column chromatography on silica with ethyl acetate/methanol 8:1. The product was recrystallized from ethanol/water. Yield 410 mg (49%) of white needles; mp. 169.5–170.8 °C; IR, ν (cm^{-1}): 3343, 2925, 2897, 2876, 2226 (CN), 1623, 1563, 1524, 1398, 1135 and 1069; ^{1}H NMR (DMSO-d_6) δ: 7.94 (2H, s, NH), 4.60 (2H, s, OH) and 3.65–3.40 (16H, m, CH_2); ^{13}C NMR (DMSO-d_6) δ: 144.2, 118.4, 116.4, 72.4, 68.1, 60.4 and 41.4.

2.1.4. 5-Diethylamino-6-(2-hydroxyethylamino)-pyrazine-2,3-dicarbonitrile (5)

A solution of **2** (1.0 g, 4.25 mmol) in tetrahydrofuran (50 mL) was stirred at r.t. and 2-aminoethanol (0.78 g, 12.75 mmol) was added dropwise. Reaction continued at r.t. for next 5 h. The mixture was then evaporated under reduced pressure, dissolved in chloroform and extracted three times with water. The organic layer was dried with Na_2SO_4, evaporated and purified by column chromatography on silica with dichloromethane/acetone 3:1 as eluent. The product was recrystallized from hot ethanol/water.

Yield 840 mg (76%) as off-white needles; mp. 102–103 °C; IR, ν (cm^{-1}): 3493, 3320, 2974, 2229 (CN), 1557, 1532, 1426, 1270 and 1049; ^{1}H NMR (acetone-d_6) δ: 6.71–6.57 (1H, br s, NH), 4.07 (1H, t, $J = 5.5$ Hz, OH), 3.79 (2H, q, $J = 5.5$ Hz, CH_2–O), 3.60 (2H, q, $J = 5.6$ Hz, CH_2–NH), 3.45 (4H, q, $J = 7.2$ Hz, CH_2–N) and 1.14 (6H, t, $J = 7.1$ Hz, CH_3); ^{13}C NMR (acetone-d_6) δ: 148.9, 148.5, 124.0, 118.7, 116.2, 116.0, 60.2, 44.5, 44.1 and 12.9.

2.1.5. 5-Diethylamino-6-[2-(4,4′dimethoxytriphenylmethoxy)-ethylamino]-pyrazine-2,3-dicarbonitrile **(6)**

A solution of **5** (418 mg, 1.6 mmol) in anhydrous pyridine (20 mL) with catalytic amount of 4-(dimethylamino)pyridine was stirred at r.t. and di(*p*-methoxyphenyl)phenylmethyl chloride (386 mg, 1.9 mmol) was added. Reaction continued at r.t. overnight. The mixture was then evaporated under reduced pressure and isolated by column chromatography on silica with toluene/pyridine 30:1. Yield 468 mg (51.8%) as yellow solid. IR, ν (cm^{-1}): 3034, 3064, 2964, 2933, 2875, 2836, 2226, 1608, 1560, 1534, 1508, 1458, 1359, 1250, 1176, 1079 and 1033; ^{1}H NMR ($CDCl_3$) δ: 7.9 (d; 2H; $J = 7.2$ Hz; aromH), 7.32–7.14 (m, 7H, aromH), 6.86–6.76 (m, 4H, aromH), 5.72 (t, 1H, $J = 5.2$ Hz, NH), 3.79 (s, 6H, OCH_3), 3.64 (q, 2H, $J = 5.1$ Hz, NHCH_2), 3.37 (t, 2H, $J = 4.9$ Hz, OCH_2), 3.32 (q, 4H, NCH_2) and 1.13 (t, 6H, $J = 7.0$ Hz, CH_3); ^{13}C NMR ($CDCl_3$) δ: 158.5, 147.4, 144.5, 135.5, 129.8, 129.1, 127.9, 127.8, 126.9, 123.5, 118.7, 115.1, 114.8, 113.1, 86.4, 60.6, 55.2, 43.4, 41.8 and 13.0.

2.1.6. 4-(2-Hydroxyethyl)-3,4-dihydro-2H-pyrazino [2,3-b][1,4]oxazine-6,7-dicarbonitrile **(8)**

Diethanolamine (630 mg, 6 mmol) was added to a solution of **1** (199 mg, 1 mmol) in tetrahydrofuran (10 mL) and the mixture was refluxed for 30 min. The solvent was evaporated under reduced pressure. The waxy solid was dissolved in dichloromethane and extracted three times with water. The organic layer was dried with Na_2SO_4, evaporated and purified by column chromatography on silica with ethyl acetate/acetone 1:1. Yield 120 mg (35.7%) of white crystals; mp. 90.0–91.3 °C. ^{1}H NMR (acetone-d_6) δ: 4.60 (t, 2H, $J = 4.6$ Hz, O–CH_2), 4.08–4.00 (m, 1H, OH), 3.95 (t, 2H, $J = 4.95$ Hz, CH_2–OH) and 3.80–3.88 (m, 4H, N–CH_2); ^{13}C NMR (acetone-d_6) δ: 149.6, 145.8, 126.2, 117.0, 115.6, 115.3, 65.6, 59.3, 51.5 and 47.5.

2.1.7. 4-Methyl-3,4-dihydro-2H-pyrazino[2,3-b][1,4]oxazine-6,7-dicarbonitrile **(9)**

2-(Methylamino)ethanol (375 mg, 5 mmol) was added to a solution of **1** (199 mg, 1 mmol) in tetrahydrofuran (10 mL) and the mixture was refluxed for 30 min. The solvent was evaporated under reduced pressure. The waxy solid was dissolved in dichloromethane and extracted three times with water. The organic layer was dried with Na_2SO_4, evaporated and purified on silica with dichloromethane/acetone 20:1. The product was recrystallized from ethanol. Yield 35 mg (17.4%) as white needles; mp. 192.3–193.5 °C. ^{1}H NMR (acetone-d_6) δ: 4.63 (t, 2H, $J = 4.8$ Hz, O–CH_2), 3.85 (t, 2H, $J = 4.8$ Hz, N–CH_2) and 3.25 (s, 3H, CH_3); ^{13}C NMR (acetone-d_6) δ: 149.6, 146.2, 126.4, 117.1, 115.6, 115.3, 65.5, 47.6 and 35.9.

2.1.8. 5,6-Bis-(2-hydroxyethylamino)-pyrazine-2,3-dicarbonitrile **(11)**

2-Aminoethanol (1.56 g, 26 mmol) was added dropwise to a solution of **1** (0.99 g, 5 mmol) in tetrahydrofuran (50 mL). This mixture was stirred for 1 h at r.t. The originated suspension was decanted from the oil drops at the bottom and solvent was removed under reduced pressure. The crude product was purified by chromatography on silica with ethyl acetate/methanol 5:1 and then recrystallized from water. Yield 1.12 g (89.5%) of white crystals; mp. 185.5–186.0 (decomposition). IR, ν (cm^{-1}): 3483, 3347, 3289, 3196, 3091, 2950, 2937, 2889, 2229 (CN), 1604, 1560, 1518, 1460, 1396, 1076 and 1060; ^{1}H NMR (DMSO-d_6) δ: 7.95 (s, 2H, NH), 4.87 (s, 2H, OH), 3.58 (t, 4H, $J = 5.5$ Hz, CH_2O), 3.44 (br s, 4H, CH_2N); ^{13}C NMR (DMSO-d_6) δ: 144.3, 118.3, 116.5, 58.7 and 44.1.

2.1.9. General procedure of synthesis of unsymmetrical AzaPc

A solution of **10** (816 mg, 3 mmol) and the second precursor (**3**, **4**, **5** or **6**) (1 mmol) in anhydrous butanol (15 mL) was heated to reflux and metal lithium (196 mg, 28 mmol) was dropped in. The mixture was heated under reflux for next 3 h. The solvent was evaporated under reduced pressure, aq. acetic acid (50% v/v, 50 mL) was added and the mixture was stirred at r.t. for 30 min. The precipitate was filtered, washed thoroughly with water and dried. This crude mixture of AzaPc was purified by column chromatography using step gradient starting from chloroform/acetone 6:1 as the first eluent to separate crude **12**. After all compound **12** was eluted from the column, the eluent was changed to a more polar one (mentioned at each preparation) and desired AzaPc was collected as very intense purple fraction. Such crude unsymmetrical AzaPc was purified by column chromatography once more, now using only the more polar eluent. The pure AzaPc was recrystallized by dissolving in minimal amount of dichloromethane and dropping into hexane. Fine suspension was filtered and dried to give pure unsymmetrical AzaPc.

2.1.10. 2-(2-Hydroxyethylamino)-3,9,10,16,17,23,24-heptakis(diethylamino)-1,4,8,11,15,18,22,25-(octaaza)phthalocyanine **(13)**

This compound was prepared following the general procedure for synthesis of unsymmetrical AzaPc starting from compounds **10** and **5**. The second eluent: tetrahydrofuran/chloroform/hexane 3:3:1. Yield 28 mg (2.6%) dark purple solid. The same results were obtained using compound **6** instead of **5**. IR, ν (cm^{-1}): 2967, 2931, 2871 and 1640; ^{1}H NMR (pyridine-d_5) δ: 13.74 (1H, br s, OH), 7.64–7.54 (1H, m, NH), 4.49–4.33 (2H, m, CH_2–OH), 4.06–3.72. (26H, m, CH_2–N + CH_2–NH), 3.63 (4H, q, $J = 6.8$ Hz, CH_2–N) and 1.30–1.05 (42H, m, CH_3); ^{13}C NMR (pyridine-d_5) δ: 152.2, 150.6, 149.1, 148.2, 144.4, 141.5, 141.0, 140.9, 137.3, 60.9, 45.0, 44.5, 43.0, 42.8 and 13.1; MS (ESI) *m/z* 1080 ($[M + H]^+$); Anal. calc. for $C_{54}H_{78}N_{24}O + 3H_2O$: C 57.22; H 7.47; N 29.67%, found C 57.04; H 7.53; N 29.59%. UV–vis λ_{max} (nm) (ε) in tetrahydrofuran: 678 (88,300), 647 (64,800), 618sh, 592sh, 507 (56,100) and 364 (104,600).

*2.1.11. 2-[2-(2-Hydroxyethoxy)-ethylamino]-3,9,10,16,17,23,24-heptakis(diethylamino)-1,4,8,11,15,18,22,25-(octaaza)phthalocyanine **(14)***

This compound was prepared following the general procedure for synthesis of unsymmetrical AzaPc starting from compounds **10** and **3**. The second eluent: tetrahydrofuran/chloroform/hexane 3:3:1. Yield 101 mg (9%) of dark purple solid. IR, ν (cm^{-1}): 2969, 2931, 2872 and 1640; ^{1}H NMR (pyridine-d_5) δ: 13.74 (1H, br s, OH), 7.43 (1H, t, $J = 5.3$ Hz, NH), 4.34 (2H, q, $J = 5.2$ Hz, CH_2–OH), 4.10 (2H, t, $J = 5.1$ Hz, CH_2–O), 4.05 (2H, t, $J = 5.1$ Hz, CH_2–O), 4.00–3.72 (26H, m, CH_2–N + CH_2–NH), 3.62 (4H, q, $J = 7.1$ Hz, CH_2–N) and 1.36–0.93 (42H, m, CH_3); ^{13}C NMR (pyridine-d_5) δ: 151.9, 150.6, 148.2, 146.6, 144.3, 141.5, 141.1, 140.9, 137.4, 73.6, 69.9, 61.7, 44.5, 43.0, 42.8 and 13.1; MS (ESI) *m/z* 1124 ($[M + H]^+$). Anal. calc. for $C_{56}H_{82}N_{24}O_2 + 3H_2O$: C 57.12; H 7.53; N 28.56% found C 57.01; H 7.56; N 28.43%. UV–vis λ_{max} (nm) (ε) in tetrahydrofuran: 677 (95,300), 647 (69,500), 617sh, 591sh, 508 (58,400) and 365 (108,000).

*2.1.12. 2,3-Bis[2-(2-hydroxyethoxy)-ethylamino]-9,10,16,17,23,24-hexakis(diethylamino)-1,4,8,11,15,18,22,25-(octaaza)phthalocyanine **(15)***

This compound was prepared following the general procedure for synthesis of unsymmetrical AzaPc starting from compounds **10** and **4**. The second eluent: chloroform/acetone/methanol 9:1:1. Dark purple solid (114 mg, 10%); IR, ν (cm^{-1}): 2968, 2930, 2871 and 1641; ^{1}H NMR (pyridine-d_5) δ: 13.63 (2H, br s, OH), 8.13 (2H, br s, NH), 4.36–4.26 (4H, m, CH_2–OH), 4.01–3.79 (32H, m, CH_2–N + CH_2–O), 3.71–3.61 (4H, m, CH_2–NH) and 1.30–1.08 (36H, m, CH_3); ^{13}C NMR (pyridine-d_5) δ: 153.5, 153.1, 151.0, 150.5, 150.4, 147.4, 143.3, 143.2, 142.8, 141.2, 139.2, 138.1, 73.3, 69.5, 61.5, 43.1, 43.0, 42.8, 42.7, 13.2, 13.1 and 13.0; MS (ESI) *m/z* 1156 ($[M + H]^+$). Anal. calc. for $C_{56}H_{82}N_{24}O_4 + 3H_2O$: C 55.61; H 7.33; N 27.80% found C 55.72; H 7.41; N 27.65%. UV–vis λ_{max} (nm) (ε) in tetrahydrofuran: 675 (86,200), 644 (67,200), 618sh, 592sh, 510 (61,300) and 363 (102,800).

2.2. Kinetic of the formation of complex with pyridine

Solution (10 mM concentration) of the chosen AzaPc (**12**, **13**, **14** or **15**) in pyridine was prepared. The solution (3 mL) was transferred into 10 × 10 mm quartz glass cell. The solution was kept in the glass cell closed with Teflon stopper during all the measurement. The first spectra were measured as soon as possible after dissolution. Dissolution time was taken as zero time; the first spectra were taken usually at 3 min.

3. Results and discussion

In the synthetic part, we have focused on AzaPc substituted with defined number (one or two) of hydroxy groups that can be modified in future with desired ligands. As mentioned above, the prerequisite for good separation of statistical mixture is low aggregation of final AzaPc molecules. The design of synthesized AzaPc followed this condition and bulky diethylamine substituents were chosen as the aggregation inhibiting moieties. That is why, they form three quarters (A, Scheme 1) of the final molecule. The hydroxy groups are placed on the last part of AzaPc macrocycle (Z, Scheme 1). Their number (one or two) depends on the substitution of the pyrazine-2,3-dicarbonitrile used as the precursor.

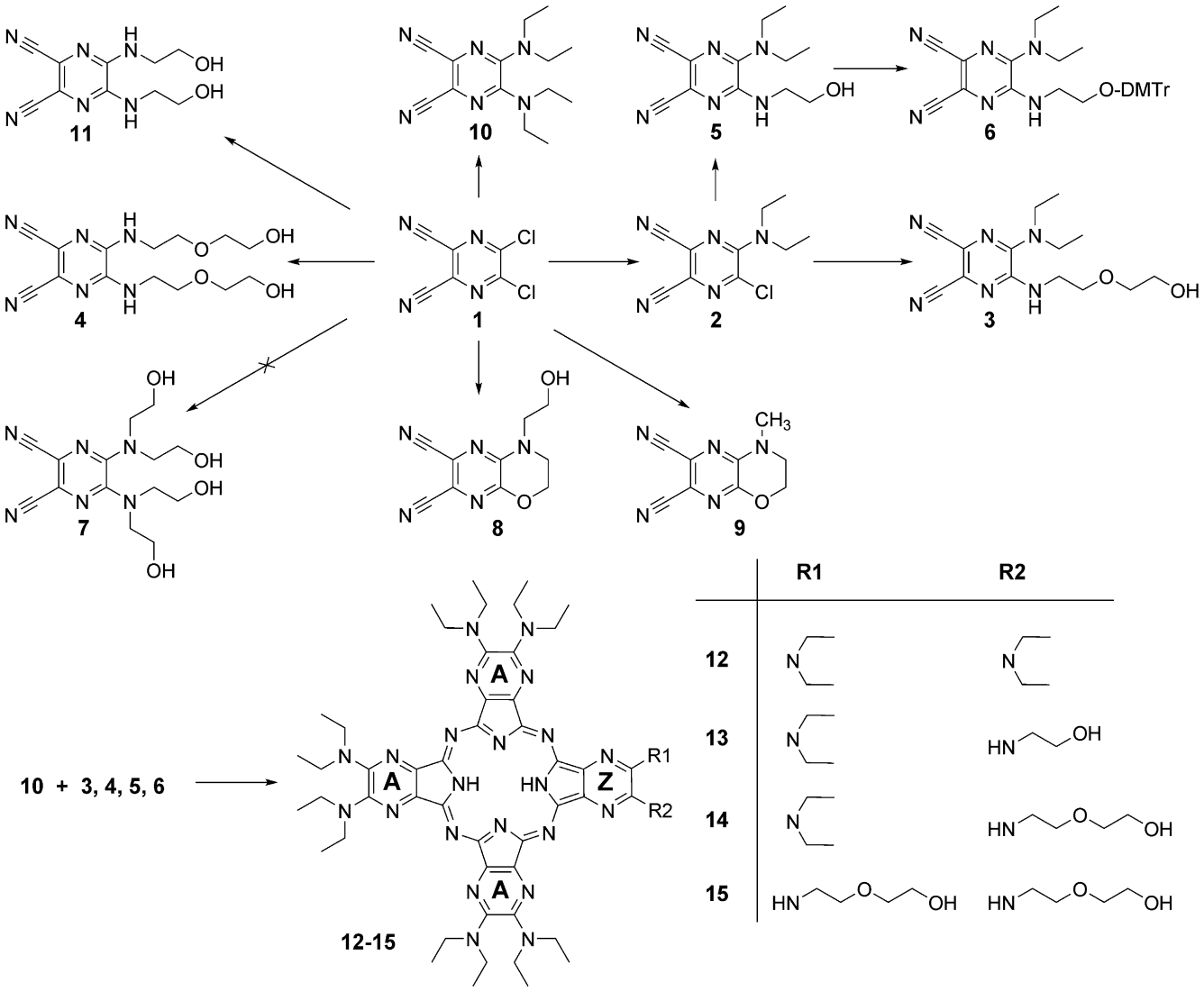
3.1. Synthesis of precursors

The synthesis of precursors for heteroatom substituted AzaPc is easiest to start from 5,6-dichloropyrazine-2,3-dicarbonitrile (**1**) (Scheme 1) that is excellent starting material for preparation of substituted pyrazine-2,3-dicarbonitriles [24–26]. Nucleophilic substitution of **1** with amines can be driven to mono- (**2**) or disubstitution (**4**, **10**, **11**) by changing the reaction conditions (stoichiometry and temperature). The second chlorine atom in **2** can be further exchanged with another substituted amine (**3**, **5**) thus leading to unsymmetrically substituted precursors with only one hydroxy group.

We have tried to prepare also precursor with four hydroxy groups (**7**) but the analysis of the main product of the reaction did not confirm this compound. Instead, the product was later characterized as a compound of different structure (**8**). Unusual formation of the morpholine ring has the following explanation (Scheme 2). The substitution of chlorine atoms in **1** with various nucleophiles [27] (amines [23], thiolates [12] or alkyloxides [28]) has been described in the literature. The first step of morpholine ring formation is therefore the substitution of one chlorine atom in **1** with strong nucleophile – secondary amine. In the next step, the free secondary amine (strong organic base) accepts hydrogen from the hydroxy group and thus it forms the alkyloxide. The alkyloxide anion (nucleophile) attacks electron-deficient carbon in the pyrazine ring next to the remaining chlorine atom. Due to bulkiness of bis(2-hydroxyethyl)amine, the possible disubstitution (the change of **1** into **7**) requires higher temperature. However, the way of closure to morpholine ring is preferred under such conditions and disables the formation of **7**. The proposed mechanism was further confirmed by synthesis of next pyrazinomorpholine derivative **9** using 2-(methylamino)ethanol.

3.2. Synthesis and characterization of the dyes

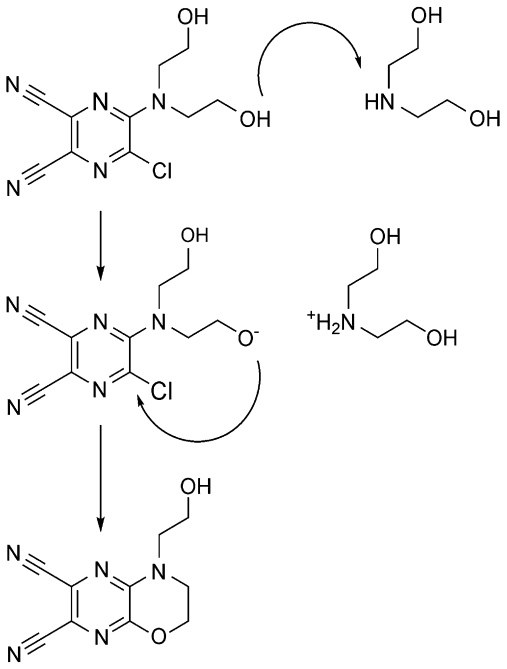
Lithium butoxide method was chosen as the cyclization procedure, since it was found formerly as the most suitable for synthesis of alkylamino substituted AzaPc [27]. The dyes of interest were synthesized using statistical condensation of compound **10** (three equivalents) with **3**, **4** or **5** (one equivalent). The ratio 3:1 was chosen as optimal. According to theoretical calculations it leads to the highest yields of AAAB type compounds when two precursors of similar reactivity are used for cyclization which is our case. In some cases, also higher ratios were used (e.g. 9:1) to facilitate the separation because AAAA and AAAB type compounds are present in the arising mixture almost exclusively [29,30]. The isolation is then easier, however, to the prejudice of the yield of AAAB type.



Scheme 1. Scheme of synthesis of precursors and AzaPc.

Due to presence of polar hydroxy groups, the R_f values of AAAB type compounds (**13**, **14**, **15**) were strongly different from other derivatives. This feature facilitated the separation and the ratio 3:1 is therefore optimal. The yield of compound **13** bearing short 2-hydroxyethylamino substituent was only 2.5% while the yields of compounds **14** and **15** with longer 2-(2-hydroxyethoxy)ethylamino substituents were both *ca* 10%. Moreover, some purple side products (usually three to four) of the same color appeared around the fraction of compound **13** indicating unexpected side reaction on periphery of macrocycle. Surprisingly, similar side products appeared also around the fraction of compound **12** isolated from the same reaction (**5** + **10**). We tried to modify the precursor **5** by blocking the hydroxyl, that might be a source of side reactions, with di(*p*-methoxyphenyl)phenylmethyl ether (dimethoxytrityl, DMTr) [31]. This protecting group is stabile in lithium butoxide (during cyclization) and is easily removed in acidic media (e.g. in 50% acetic acid used to remove the rests of lithium). Compound **6** protected with DMTr, however, did not improve the yield after cyclization. The purity of the fractions remained also the same as in the case of non-protected **5** so the hydroxy group does not play an important role in the induction of such side reaction. Unfortunately, we did not succeed in identifying of the side products or the mechanism leading to them. On the other hand, it may be concerned with presence of 2-hydroxyethylamino moiety. Compound **11** with two of these substituents did not give any unsymmetrical derivatives in statistical condensation with **10**. Only compound **12** was always detected using different methods of cyclization. Furthermore, **11** did not even tetramerize itself to symmetrical AzaPc, only brown decomposition products were observed.

The hydroxy groups are suitable substrates for binding to other ligands through ester or ether bonds. Just to check it, such possibility was tested in simple reactions which were monitored only on TLC. Compound **14** was acetylated using acetic anhydride in THF with small amount of pyridine. Compound **14** was also etherified with DMTr in anhydrous pyridine. TLC showed in both cases full transformation to compounds of much higher lipophilicity. DMTr ether of **14**



Scheme 2. The mechanism of morpholine ring formation.

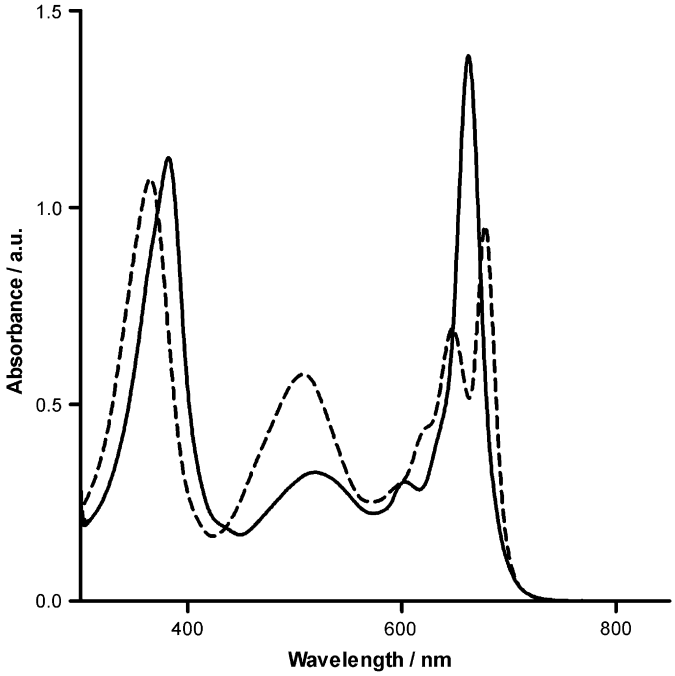


Fig. 1. UV–vis absorption spectra of compound **14** in THF (dashed line) and in pyridine (full line) after full formation of the complex with two pyridine molecules.

is not stabile under acidic conditions and it releases again compound **14** with free hydroxy group. The reactivity of hydroxyls is therefore not influenced in the AzaPc macrocycle.

All compounds **12–15** were characterized using typical analytical methods – IR, NMR, MS, elementary analysis and UV–vis. All of them confirmed the identity and purity of isolated compounds. Compound **12** which was isolated from the reactions was compared with the same compound prepared before [21,32] by simple tetramerization of only **10** and the identity was confirmed.

All compounds showed similar UV–vis absorption spectra typical for Pc or AzaPc (Fig. 1, solvent THF). Absorption at approximately 376 nm is attributed to B band and its strength is typical for AzaPc of tetrapyrazinoporphyrazine type [27]. Absorption in the region around 510 nm arises from the n–π* transitions of the lone pair electron in non-bonding orbital of nitrogen connecting peripheral chains to macrocycle. Similar observations were made also for compounds bearing oxygen [33,34] or sulphur [21] directly attached to Pc ring. The Q-band of all AzaPc is split due to lower symmetry of the macrocycle (D_{2h}) caused by presence of two central hydrogens. The symmetry of the macrocycle was not lowered further with different peripheral substituents because all of them exert almost the same electronic effects. No aggregation was observed up to concentration *ca* 50 μM in THF (the maximum used) due to bulky peripheral diethylamino substituents. The aggregation decreases extinction coefficients in Q-band area and modifies its shape [35] but no such changes were observed.

3.3. Formation of complexes with pyridine

Metal-free Pc [36] or AzaPc [21,37] macrocycles behave as weak *N*-acids and can undergo a deprotonation in basic media on both central nitrogens (N^{29} and N^{31}). This process is usually accompanied by a change of color. In case the protons are completely removed (e.g. as molecules of water in the case of reaction with tetrabutylammonium hydroxide [21]), a true ionic compound originates. However, a formation of so-called ''proton-transfer complex'' has been suggested in several works in the case of interaction of metal-free Pc or porphyrazines with weak bases (amines, DMSO and DMF) [38,39]. It proceeds according to following reaction (example for pyridine):

$$H_2AzaPc + 2\ pyridine \rightarrow pyridine\ldots H\ldots AzaPc\ldots H\ldots pyridine \quad (1)$$

As it has been shown before also by us for AzaPc, the molecule does not loose the hydrogen completely but it forms adducts with pyridine [21]. In this work, we have investigated also the kinetics of the complex formation of prepared AzaPc **12–15**. UV–vis absorption spectra of AzaPc slowly changed after dissolution in pyridine (Fig. 2). This process was accompanied by change in color from purple to blue. The split Q-band that characterizes D_{2h} symmetry slowly changes to unsplit typical for D_{4h} symmetry (Fig. 1, solvent pyridine). The D_{4h} spectrum is typical for highly symmetric AzaPc macrocycles and can be obtained only when both molecules of pyridine are complexed, each on one side of the macrocycle. Perfect isosbestic points were observed during measurements (Fig. 2), thus indicating that the adduct is formed directly with two molecules of pyridine, without detectable amount of intermediate with one molecule.

The studied AzaPc **12–15** showed different kinetic rates of the complex formation. The reaction (Eq. (1)) runs under pseudo-first order kinetics because pyridine – one of the

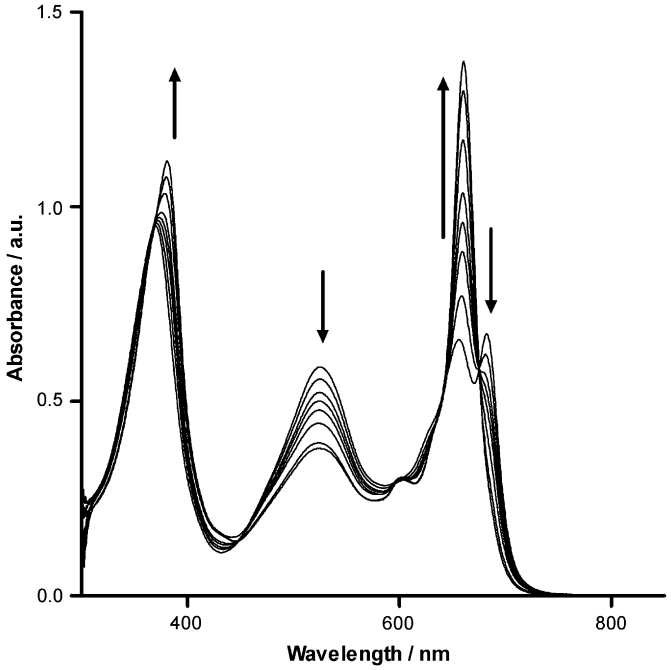


Fig. 2. Changes of UV–vis absorption spectra of compound **15** in pyridine with time (from 3 min to 24 h).

reactants – is in large excess and therefore its concentration remains constant. In such a case Eq. (2) can be derived.

$$A_t = A_0 + (A_{max} - A_0)\left(1 - e^{-k_r t}\right) \quad (2)$$

where A_t is absorbance in time t, A_{max} is absorbance in the plateau region, A_0 is absorbance at the zero time and k_r is rate constant. The changes were monitored at fixed wavelength, usually at the Q-band maximum of the formed complex (around 662 nm, see Table 1) where the changes in absorbance are the highest. The rate constant can be derived using non-linear regression with data from several different times (Fig. 3). Best fitted values of k_r as well as UV–vis spectral data are summarized in Table 1.

As it is obvious from the rate constants k_r, the adduct formation was much slower (almost one order) in the case of compounds **13** and **15**. We have performed also the studies of compound **12** (no hydroxy group in the structure) in pyridine with addition of alcohols (ethanol, *n*-octanol – always 1%, v/v) to confirm or refuse the effect of hydroxy groups on the speed of

Table 1
Constant rates k_r of the pyridine complex formation and the spectral data of the prepared compounds **12**–**15** in Q-band area

Compound	k_r (s^{-1})	λ_{max} (ε) in THF	λ_{max} (ε) of the complex in pyridine[a]
12	1.62×10^{-4}	679 (93,700), 649 (68,600)	663 (131,500)
12 + ethanol	2.45×10^{-4}	–	663 (151,400)
12 + *n*-octanol	1.82×10^{-4}	–	663 (150,700)
13	3.00×10^{-5}	678 (88,300), 647 (64,800)	662 (108,800)
14	1.71×10^{-4}	678 (95,300), 647 (69,500)	662 (138,600)
15	6.05×10^{-5}	675 (86,200), 644 (67,200)	661 (137,300)

[a] Data taken in plateau region of the kinetic analysis.

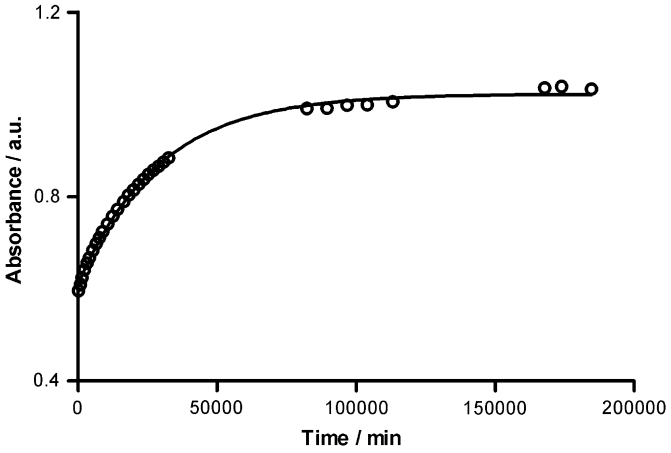


Fig. 3. Absorbance of the pyridine solution of **13** at 662 nm in dependence on time after dissolution. Measured values (○) and calculated values (line) according to Eq. (2) with best fitted value of $k_r = 3.00 \times 10^{-5}\ s^{-1}$.

formation of the proton-transfer complex with pyridine. The reaction rates were comparable with or even slightly higher than in the case when no alcohols were present. The hydroxy groups therefore do not influence the speed of this reaction and the differences arise only from the other structural factors.

4. Conclusion

We have synthesized several new precursors for AzaPc bearing hydroxy group. During synthesis of precursors we have observed unusual formation of new morpholine ring and we have explained the mechanism leading to it. Synthesis of AzaPc using statistical condensation gave desired products of AAAB type which were successfully isolated and characterized. Isolated AzaPc are substituted with defined number (one or two) of modifiable hydroxy groups. This makes them suitable for conjugation with one or two ligands in future. Slow change of solution color of these metal-free AzaPc in pyridine is due to formation of proton-transfer complex with two molecules of pyridine. The complex is formed directly with two molecules of pyridine, the presence of intermediate with only one pyridine molecule was not observed. The rate constant of this process is not the same for all compounds and may depend on the structure of macrocycle. The influence of hydroxy groups was not confirmed and they may not play a role.

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