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Synthesis of β−Octaethylporphyrin Conjugates with Nitrogen and Sulfur Containing Heterocycles

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Conjugates of Ni(II) β*-octaethylporphyrin with nitrogen and sulfur containing heterocycles were obtained by meso-functionalization of the porphyrin. The meso-functionalization of Ni(II)* β*-octaethylporphyrin was performed by the Vilsmeier-Haack formylation reaction, followed by the Knoevenagel reaction of Ni(II) meso-formyl-*β*-octaethylporphyrin with CH-acids: malonic ester, thiohydantoin, and thiobarbituric acid. The product of condensation of the porphyrin with malonic ester was cyclized with hydrazine to the corresponding porphyrin conjugate with pyrazolidine-3,5-dione. The optical spectra of the obtained new dyes are modified compared to that of the initial compounds. The spectral changes of the porphyrin conjugate with thiobarbituric acid are particularly dramatic reflecting the considerable interaction between tetrapyrrolic and heterocyclic chromophores in the molecule.*

Keywords: Porphyrins, heterocycles, Knoevenagel condensation, thiohydantoin, thiobarbituric acid, malonic ester.

Синтез конъюгатов β−октаэтилпорфирина с азот− и серосодержащими гетероциклами

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Путем мезо-функционализации Ni(II) комплекса β*-октаэтилпорфирина получены его конъюгаты с азот- и серосодержащими гетероциклами. Для этого была использована реакция формилирования Вильсмейера-Хаака с последующей реакцией Кневенагеля Ni(II) мезо-формил-*β*-октаэтилпорфирина с СН-кислотами: малоновым эфиром, тиогидантоином и тиобарбитуровой кислотой. Циклизация продукта конденсации порфирина с малоновым эфиром с помощью гидразина привела к соответствующему конъюгату порфирина с пиразолидин-3,5-дионом. Оптические спектры полученных новых красителей модифицированы по отношению к спектрам исходных соединений. Спектральные изменения конъюгата порфирина с тиобарбитуровой кислотой особенно драматичны, отражая значительное взаимодействие между тетрапиррольным и гетероциклическим хромофорами в молекуле.*

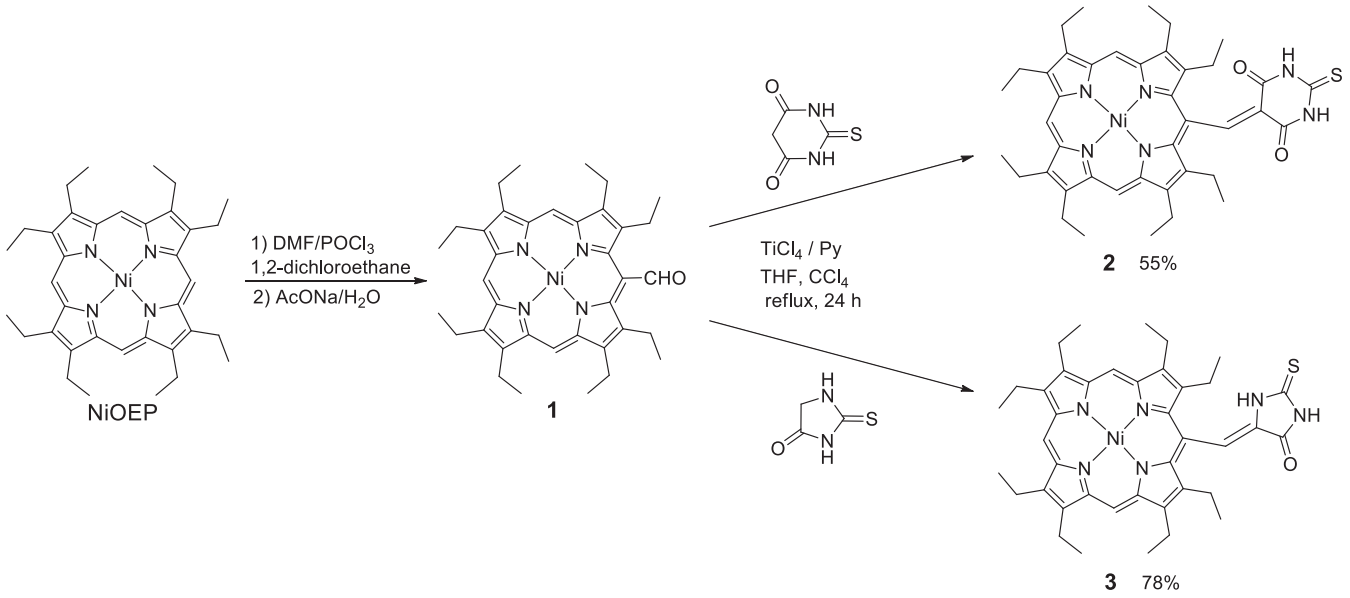
Ключевые слова: Порфирины, гетероциклы, конденсация Кневенагеля, тиогидантоин, тиобарбитуровая кислота, малоновый эфир.

Recognition and analysis of compounds in various environments is a central task of analytical chemistry, and many other applied fields ranging from environmental control to clinical diagnostics depend on the solution of that problem which needs the development of new methods. The modern direction of the development of analytical methods lies in a field of sensors being simple devices that can provide real-time information about the presence of specific compounds in complex samples.[1] One of the areas of sensor systems is optical sensing which allows remote and non-contact analysis by non-destructive methods, which is extremely important for the purposes of medical diagnostics. Using such sensors, oxygen and pH concentrations can be measured in cells and tissues, thus evaluating their bioenergetics,[2,3] which makes it possible to diagnose many diseases, in particular, tumors.[4,5] The key elements of optical sensors are sensor dyes capable to change their photophysical characteristics upon interaction with an analyte.[1] Porphyrins and other related tetrapyrrole macrocycles are widely used as such indicator dyes.[6–9] To make the dye sensitive to the analyte, specific functional groups are to be inserted into the porphyrins.[6] Among a variety of sensor fragments we have selected nitrogen and sulfur containing heterocycles: thiohydantoin, thiobarbituric acid (TBA) and pyrazolidine-3,5-dione. These heterocycles possess p*H* sensitivity, can coordinate metal cations, and also possess biological activity[10,11] and are able to interact with cellular receptors.[12–14] Metal coordination ability led to the use of thiohydantoin and TBA derivatives in the analysis of cations[15,16] and anions.[17] Thus, combinations of porphyrin dyes with such heterocyclic sensor fragments can be of interest as potential promising sensor dyes.

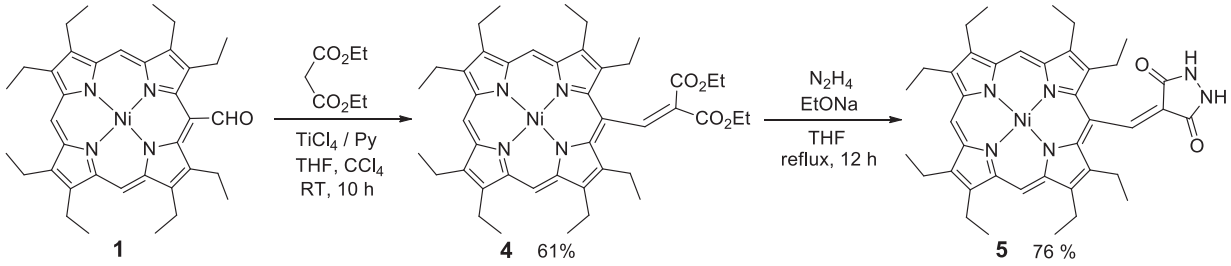
β-Octaethylporphyrin (OEP) being one of the most used model porphyrins, along with *meso*-tetraphenylporphyrin, was chosen as the primary substrate for the synthesis of porphyrin conjugates with heterocycles. Since OEP is closer in structure to natural porphyrins, in which all β-positions are substituted, and *meso*-positions are free, it is this porphyrin that is often used to model natural ones. The free *meso*-positions of the aromatic tetrapyrrole macrocycle have an increased reactivity and easily react with electrophiles in electrophilic substitution reactions. That allows easily to insert substituents into the macrocycle. The primary functionalization of OEP was carried out using the Vilsmeier–Haack formylation reaction.[18] For this purpose, an OEP complex with Ni(II) (NiOEP) was first obtained, since metal complexes are more active in electrophilic reactions due to an increase in the electron density in the tetrapyrrole ring, and nickel complexes are sufficiently resistant to protodemetallation in acidic media under the conditions of the formylation reaction. When the nickel complex is formylated with the Vilsmeier reagent obtained *in situ* by the interaction of *N,N*-dimethylformamide and phosphorus(V) oxochloride, a "phosphorus complex" is formed, the hydrolysis of which leads to Ni(II) *meso*-formyl-β-octaethylporphyrin **1**.[19] The porphyrin with aldehyde group, which is easily accessible by this way, is a versatile building block,[20] often used in diverse transformations inherent to the carbonyl group,[21] in particular, in oxidation, reduction, formation of oximes, semicarbazones, imines and cyanohydrins, condensation with CH-acids, Wittig reaction, as well as reactions with organometallic reagents.[22–24] Previously, we have reported on the functionalization of the porphyrins through the formylation, followed by the condensation with nitrogen nucleophiles, leading to insertion of imino,[25–27] hydrazono[19,28] and azino[29] groups into the *meso*-position of the tetrapyrrole macrocycle. In the framework of this work, the formylporphyrin was used in condensation reactions with CH-acids to obtain porphyrin conjugates with heterocycles. Condensation of aromatic aldehydes with TBA catalyzed by hydrochloric acid was described more than 100 years ago,[30] However, the reaction has not been studied with porphyrin substrates. Aqueous hydrochloric acid is not suitable for insoluble in water porphyrin substrates, and *meso*-formyl group is less reactive due to the sterical hindrances from nearby β-pyrrolic ethyl substituents, hence we searched for alternative catalysts. For the condensation of formylporphyrin with malonic ester, titanium tetrachloride was previously used as a catalyst and the reaction was carried out in pyridine.[31] This catalyst was applied in the current work for condensation of **1** with thioheterocycles. The reaction of the formylporphyrin **1** with TBA was carried out in refluxing mixture of THF and CCl_4 using $TiCl_4$/Py as a catalyst. As a result, the conjugate of the NiOEP with TBA with bridging C=C double bond **2** was formed with 55 % yield (Scheme 1).† Using the same elaborated procedure, condensation of the formylporphyrin **1** with thiohydantoin was performed, and the corresponding conjugate of porphyrin with thiohydantoin **3** was obtained with 78 % yield.

An alternative way of the conjugate synthesis is a functionalization of the porphyrin followed by the transformation of the appropriate functional group to a heterocycle moiety. Exploring this way, the condensation of **1** with malonic ester was carried out first, obtaining porphyrinylmethylenemalonate **4**,[31] Then, the malonic

† A solution of $TiCl_4$ (5.5 mL, 50 mmol) in 12 mL of dry CCl_4 was added dropwise to rapidly stirred dry THF (250 mL) at 0 °C. The resulting mixture was stirred for 5 min, then the solution of Ni(II) 5-formyl-2,3,7,8,12,13,17,18-octaethylporphyrin **1** (50 mg, 0.08 mmol) and a solution of a CH-acid (1.61 mmol) in 12 mL of dry THF was added to the reaction mixture. After that the solution of dry pyridine (8 mL, 99 mmol) in 15 mL of dry THF was added dropwise to the reaction mixture at 0 °C over 3.5 h, and the resulting mixture was stirred at reflux for 24 hours. Then water (30 mL) and methyl *tert*-butyl ether (30 mL) were added to a reaction mixture, the organic phase was separated and washed with water (4×30 mL), dried over anhydrous sodium sulphate, evaporated in vacuum and the product was purified with preparative TLC in dichloromethane : petroleum ether = 7 : 3. Compound **2**. Yield 33 mg (55 %) (brown amorphous solid). ^{1}H NMR (600 MHz, $CDCl_3$, 303 K) δ_H ppm: 10.24 (1H, s, 5^1-CH), 8.82 (1H, s, 15-CH), 8.73 (2H, s, 10, 20-CH), 3.49 (16H, m, C*H*$_2$CH$_3$), 2.05 (1H, s, NH), 1.62 (18H, m, CH$_2$C*H*$_3$), 1.55 (6H, m, CH$_2$C*H*$_3$). ^{13}C NMR (150 MHz, $CDCl_3$, 303 K) δ_H ppm: 9.45 (3H, s, 10, 15, 20-CH), 8.89 (1H, s, 5^1-CH), 8.37 (1H, s, NH), 6.80 (1H, s, NH), 3.82 (16H, m, C*H*$_2$CH$_3$), 1.80 (12H, m, CH$_2$C*H*$_3$), 1.72 (12H, m, CH$_2$C*H*$_3$). ^{13}C NMR (150 MHz, $CDCl_3$, 303 K) δ_C ppm: 174.8 (C=S), 162.2 (C=O), 146.9, 144.0, 143.5, 143.3, 140.7, 140.5, 138.4 and 138.0 (pyrrole cycles), 131.8 (5^1-CH), 112.8 (5^2-C), 100.4 (5-C), 98.1 (10, 20-CH), 97.8 (15-CH), 22.1, 19.59, 19.54 and 19.50 (CH_2), 18.17, 18.09, 18.02 and 17.3 (CH_3). UV-Vis (CH_2Cl_2) λ_{max} (relative absorption) nm: 402 (1.00), 530 (0.10), 566 (0.15). LDI TOF *m/z*: found 717.14, calc. for $[M+H]^+$ $C_{40}H_{47}N_6NiOS$ 717.29.



Scheme 1. Synthesis of the Ni(II) β-octaethylporphyrin conjugates with thiohydantoin and TBA.



Scheme 2. Synthesis of the Ni(II) β-octaethylporphyrin conjugate with pyrazolidine-3,5-dione.

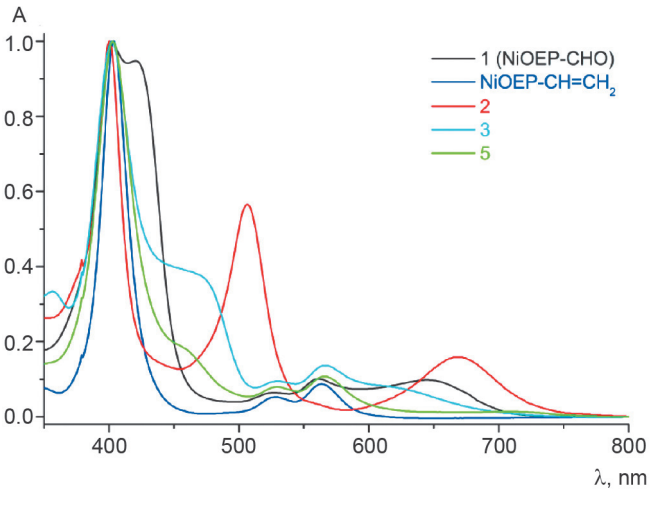


Figure 1. UV-Vis spectra of the functionalized NiOEP derivatives: starting *meso*-formyl-β-octaethylporphyrin **1** (NiOEP-CHO), *meso*-ethenyl-β-octaethylporphyrin (NiOEP-CH=CH_2) and synthesized conjugates of NiOEP with heterocycles **2**, **3**, **5**. Spectra were recorded in CH_2Cl_2 at concentration of 10^{-5} M.

ester functional group of the product **4** was cyclized with hydrazine using the reported procedure[32] of refluxing in absolute alcohol in the presence of sodium ethylate.‡

‡ To a solution of **4** (20 mg, 0.03 mmol) in 3 mL of ethanolic sodium ethoxide solution (0.1 g Na in 30 mL of absolute ethanol) hydrazine hydrate was added, and the resulting mixture was stirred under reflux for 10 hours. After reaction completion the solvent was evaporated, the residue was dissolved in 10 mL of CH_2Cl_2 and washed with water (3×10 mL), dried over anhydrous sodium sulphate, evaporated in vacuum and the product was isolated with preparative TLC in eluent CH_2Cl_2 : MeOH : Et_3N = 20 : 1 : 0.05, yielding 14 mg (76 %) of compound **5** (pink amorphous solid). ^{1}H NMR (600 MHz, $CDCl_3$, 303 K) δ_H ppm: 11.05 (1H, s, 5^1-CH), 10.46 (2H, s, NH), 9.48 (2H, s, 10, 20-CH), 9.47 (1H, s, 15-CH), 3.85 (12H, m, CH_2CH_3), 3.73 (4H, m, CH_2CH_3), 1.79 (12H, m, CH_2CH_3), 1.71 (6H, t, CH_2CH_3), 1.52 (6H, t, CH_2CH_3). ^{13}C NMR (150 MHz, $CDCl_3$, 303 K) δ_C ppm: 168.4 (C=O), 165.6 (C=O), 155.2 (5^1-CH), 145.2, 143.8, 143.2, 143.0, 140.0, 139.6, 138.9 and 136.8 (pyrrole cycles), 127.7 (5-C), 108.4 (5^2-C), 96.9 (10, 20-CH), 95.7 (15-CH), 22.0, 19.6, 19.5 and 19.4 (CH_2), 18.3, 18.1 and 16.2 (CH_3). UV-Vis (CH_2Cl_2) λ_{max} (relative absorption), nm: 403 (1.00), 529 (0.07), 566 (0.11). LDI TOF *m/z*: found 701.33, calc. for $[M+H]^+$ $C_{40}H_{47}N_6NiO_2$ 701.31.

As a result, a porphyrin conjugate with pyrazolidine-3,5-dione **5** was formed with an yield of 76 % (Scheme 2).

The UV-Vis spectra (Figure 1) of the obtained NiOEP conjugates with heterocycles **3**, **5** do not significantly differ from that of the structurally similar porphyrin chromophore *meso*-vinyl substituted NiOEP (NiOEP-CH=CH_2). The only change is the appearance of the shoulder at 460–470 nm which originates from the methylene substituted heterocyclic chromophore. The little changes of the bands of the porphyrin chromophore is a consequence of the absence of the conjugation of the π-electrons of the double bond linker with aromatic tetrapyrrolic macrocycle due to the near orthogonal turn of the *meso*-substituted double bond relatively to the plane of the macrocycle. This turn is caused by the steric interactions of the heterocycle with β-pyrrolic substituents, which is common for *meso*-ethenyl substituted porphyrins.[33,34] Nevertheless, the sensor properties of the conjugates may rely on the increased interactions between the porphyrin dye and the heterocycle receptor in the excited state.[33,35] The dramatic spectral change of the conjugate with TBA **2** was quite different. The new strong absorption band arose at 509 nm. Thiobarbituric chromophore itself absorbs in UV region only, and structurally similar benzylidene substituted TBA has a longest wavelength band at 358 nm. However, TBA adduct with malonic aldehyde exhibits strong band at 532 nm.[36] Thus, the band at 509 nm might be associated with a methylene-substituted TBA fragment. The porphyrin Q-band was red shifted for tremendous hundred nm up to 669 nm, and this value is not typical for porphyrins, rather, it is inherent to natural chlorins such as chlorophyll *a*.[37,38] That allows to suggest existence of the considerable electronic interaction between the porphyrin and TBA parts of the conjugate **2**. The reason of such difference between **2** and other conjugates is to be investigated.

In conclusion, we have prepared new porphyrin compounds, namely conjugates of nickel complex of β-octaethylporphyrin with nitrogen and sulfur containing heterocycles: thiobarbituric acid, thiohydantoin, and pyrazolidine-3,5-dione. The elaborated synthetic method allows to obtain valuable porphyrin-heterocycle derivatives with good yields from the readily accessible β-octaethylporphyrin in a straightforward way using low cost reagents. The optical spectra of the compounds contain new bands arose from the interaction of the conjugated chromophores as well as bathochromically shifted original absorption bands. Particularly dramatic changes were observed in the spectrum of the porphyrin conjugate with thiobarbituric acid, which led to substantial increase of absorption in green and red spectral region.

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