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Conjugates of natural chlorins with cyclen as chelators of transition metals

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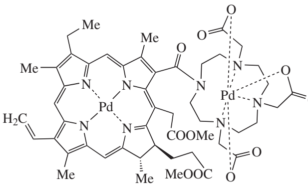
Novel herein synthesized conjugate of chlorin e_6 methyl ester with cyclen, as well as its mono- and dipalladium complexes, are regarded in view of their use in therapy and diagnostics.

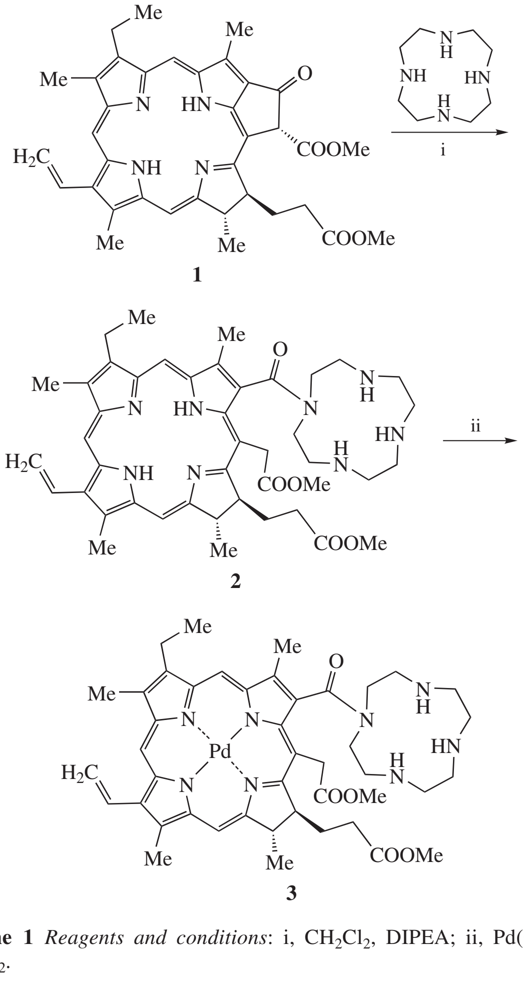


Currently, studies are under way aimed at the preparation of macrocyclic complexons based on conjugates of porphyrins and their hydrogenated analogues with aza-crown ethers, cyclams, cyclens, *etc.*, which are capable of additional complexing metal cations.[1] The availability of two coordination cavities in such conjugates makes it possible to obtain homo- and heteronuclear metal complexes which may be in demand in fluorescent diagnostics,[2–4] magnetic resonance tomography and positron emission tomography.[5] Application of such contrast agents allows one to study biological tissues at various depths with high resolution, which considerably improves the efficiency of early cancer diagnostics.[6] The complex of 1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid with Gd^{3+} cation possessing a high magnetic moment and a long relaxation time of electron spins, and the complex of porphyrin with the ^{64}Cu radioactive isotope find such a usage.[7] The affinity of porphyrins and related compounds to tumor tissues improves the diagnostic capabilities of heteronuclear metal complexes of this kind.[8–10] Taking into account that incorporation of Pd^{2+} cation enhances the photosensitizing activity of porphyrin–chlorin compounds,[11,12] it can be expected that heterometallated cyclen–chlorin conjugates would manifest the properties of theranostics that combine therapeutic efficiency with options for non-invasive diagnostics in oncology.

In this study, we obtained derivatives of natural chlorophyll *a* with cyclen as possible precursors for incorporation of metal atoms into the coordination spheres of macrocycles for implementation of various kinds of diagnostics.

Pheophorbide *a* methyl ester **1** was used as the key compound in syntheses of conjugates with cyclen. It is obtained from chlorophyll *a* contained in *Spirulina platensis* cyanobacteria using the technique that we suggested previously.[13] The conjugate was synthesized by the direct reaction of compound **1** with cyclen. Cyclen acts as a nucleophile which opens the pentanone exocycle with cleavage of the C^{13^1}–C^{13^2} bond (Scheme 1). The ease of exocycle opening is due to the availability of a sterically accessible nucleophilic center at the C^{13^1} carbon atom.

The actual experiments were preceded by quantum-chemical calculations of the product using G09W software without setting additional conditions for conformational analysis.[14] The structure of the conjugate of pheophorbide *a* methyl ester with cyclen in chloroform was optimized using the spin-limited B3LYP density functional in 6-311G++ basis. The starting geometry was found using the force field of MM+ molecular mechanics. The appearance of a spatial steric factor due to the interaction of the macrocyclic moiety possessing high flexibility and conformational mobility, on the one hand, and the tetrapyrrole macrocycle with a rigid skeleton and an invariable size of the internal cavity, on the other hand, was observed (Figure 1).



Scheme 1 *Reagents and conditions*: i, CH_2Cl_2, DIPEA; ii, $Pd(OAc)_2$, CH_2Cl_2.

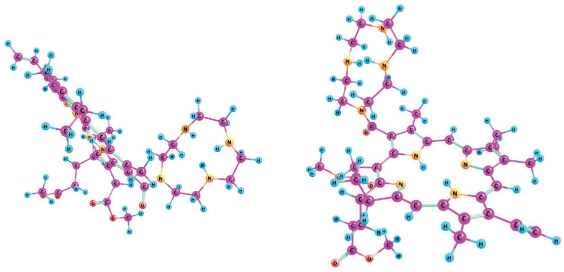


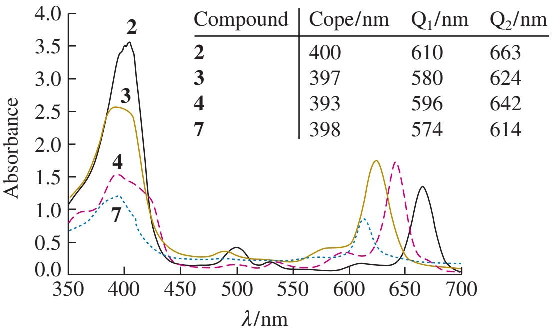
Figure 1 Principal structure of conjugates of pheophorbide *a* methyl ester with cyclen obtained after calculation of the RB3LYP/6-311G++ global minimum.

Taking into consideration binding of two subunits upon formation of an amide bond, which results in restriction of conformational mobility, as well as steric factor, we expected a low conversion of pheophorbide *a* methyl ester in this reaction. Our previous experience in opening the pentanone ring in pheophorbide and in bacteriopheophorbide with various amines has shown that the nucleophile structure affects the reaction duration and the yield of the reaction product.[15] However, the yield of compound **2** in this study was rather high (77%). The course of the reaction was monitored chromatographically, based on a considerable decrease in the R_f of the reaction product **2** in comparison with pheophorbide methyl ester **1**.[†]

The structure of the target conjugate was confirmed by the ^{1}H NMR spectrum containing characteristic signals of protons in pheophorbide *a* methyl ester moiety that coincide with literature data,[16] while the presence of a cyclen moiety in the compound was confirmed by the presence of proton signals in the 3.0–2.0 ppm region.

Incorporation of metal cations in the chlorin macrocycle fundamentally changes the properties of the resulting ensemble and is a prerequisite in the development of contrast agents for diagnostics. It is known that in the porphyrins–chlorins–bacteriochlorins series, the bathochromic shift of the main absorption band Q is accompanied by a decrease in the own fluorescence of these compounds. Incorporation of certain *d*-metals, including Pt and Pd,[17–20] in the chlorin macrocycle can favor phosphorescence buildup, which favors the diagnostic capabilities of photosensitizers of porphyrin nature.

Reaction of conjugate **2** with palladium acetate was carried out under mild conditions at room temperature (see Scheme 1). The course of the reaction and formation of metal complex **3** were monitored by the hypsochromic shift of the absorption band from 663 to 624 nm (Figure 2).[‡]



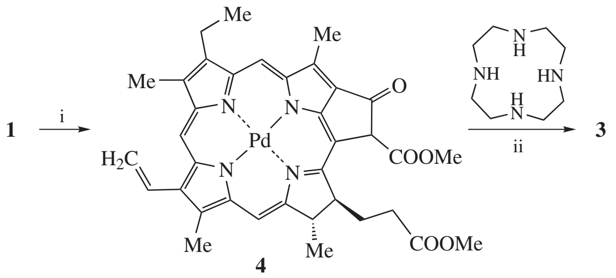
Compound	Cope/nm	Q_1/nm	Q_2/nm
2	400	610	663
3	397	580	624
4	393	596	642
7	398	574	614

Figure 2 Absorption spectra of compounds **2**–**4**, **7**.

Though incorporation of a metal into cyclen during the metallation of conjugate **2** is unlikely due to a non-planarity of the tetraaza macrocycle, we had to prove that the palladium cation is regioselectively incorporated exactly into the chlorin macrocycle. With this in mind, we first obtained a Pd complex of pheophorbide methyl ester **4** with a long-wave absorption band Q_2 = 642 nm, which was then reacted with cyclen (Scheme 2).[§] The conjugate obtained in the alternative manner was identical to the metal complex **3** in terms of spectral properties and chromatographic mobility (see Figure 2).

The presence of a palladium cation in conjugate **3** is clearly confirmed by the presence of a group of signals corresponding to the molecular ion with the main palladium isotopes in the MALDI mass spectrum.

To implement the chelating properties of cyclen, an additional modification of the tetraaza macrocycle is required, namely, incorporation of acetic acid residues at the secondary nitrogen atoms. The described in the literature direct alkylation of cyclen with chloro- or bromoacetic acid proved to be unsuccessful in our system, since the resulting mono-, di- and trisubstituted derivatives are difficult to separate due to similar chromatographic mobilities. Therefore, we carried out alkylation of conjugate **2** with *tert*-butyl bromoacetate[21,22] in dichloromethane in the presence of DIPEA. The course of the reaction was monitored chromatographically, based on the increase in the R_f of product **5**



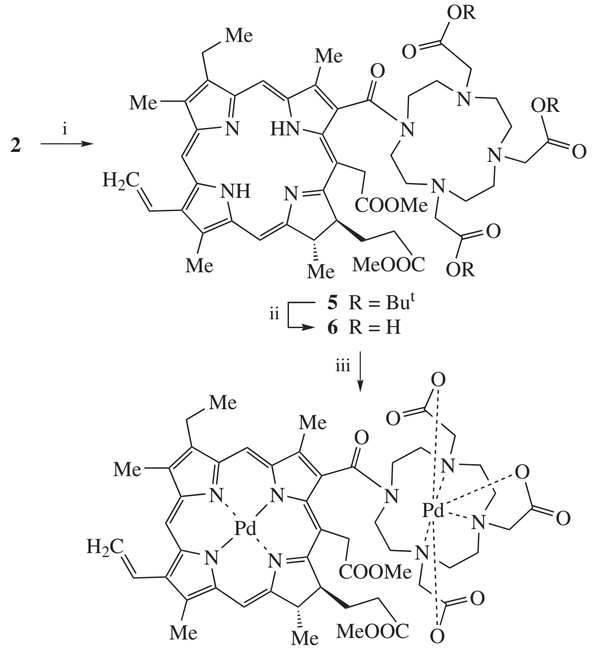
Scheme 2 *Reagents and conditions*: i, $Pd(OAc)_2$, CH_2Cl_2; ii, DIPEA, CH_2Cl_2.

[†] *Conjugate of chlorin* e$_6$ *dimethyl ester with cyclen* **2**. Crystals of cyclen (5.8 mg, 0.048 mmol) were added to a solution of pheophorbide *a* methyl ester **1** (15 mg, 0.025 mmol) in dichloromethane (5 ml) with DIPEA (0.1 ml). The mixture was stirred at room temperature for 12 h (TLC monitoring), then transferred into a separating funnel and extracted with dichloromethane until complete discoloration of the dichloromethane layer (5×30 ml). The extracts were combined, dried with anhydrous sodium sulfate and concentrated. The product was purified by preparative chromatography in the dichloromethane–methanol (0.7%) system to afford 77% of the target compound. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.76 (s, 1H, *meso*-H^{10}), 9.69 (s, 1H, *meso*-H^5), 8.83 (s, 1H, *meso*-H^{20}), 8.10 (t, 1H, C*H*=CH_2), 6.38 (dd, 1H, *trans*-3-CH=C*H*$_2$), 6.18 (dd, 1H, *cis*-3-CH=C*H*$_2$), 4.68–4.50 (m, 2H, H^{17}, H^{18}), 4.30 (m, 3H, 15-CO_2Me), 4.10 (s, 2H, 15^1-CH_2), 3.81–3.75 (m, 2H, 8-C*H*$_2$Me), 3.65 (s, 3H, 17-$CH_2CH_2CO_2$*Me*), 3.52, 3.46, 3.33 (s, 3×3H, 2,7,12-Me), 2.94–2.78 (m, 4H, 17-C*H*$_2$C*H*$_2$$CO_2$Me), 2.42–1.97 (m, 16H, cyclo-CH_2), 1.76 (m, 6H, NH, 18-Me), 1.65 (t, 3H, 8-CH_2*Me*), 0.75 (br. s, 1H, NH), –1.68 (br. s, 1H, NH). UV (λ_{max}/nm): 400 (Soret), 610, 663. MS (MALDI TOF/TOF), *m*/*z*: 779.460 [M+1]$^+$.

[‡] *Conjugate of Pd complex of chlorin* e$_6$ *dimethyl ester with cyclen* **3**. Palladium acetate (1.1 mg, 0.005 mmol) was added to a solution of conjugate **2** (2.3 mg, 0.003 mmol) in dichloromethane (0.5 ml). The mixture was stirred for 8 h. An increase in TLC chromatographic mobility was observed. After completion of the reaction, the solvent was concentrated *in vacuo*. The product was purified and crystallized in a dichloromethane–hexane system. Yield, 98%. UV (λ_{max}/nm): 397 (Soret), 580, 624. MS (MALDI TOF/TOF), *m*/*z*: 882.169 (^{105}Pd, [M+1]$^+$), 883.189 (^{106}Pd, [M+1]$^+$), 885.175 (^{108}Pd, [M+1]$^+$).

[§] *Pd complex of pheophorbide* a *methyl ester* **4**. Pheophorbide *a* methyl ester **1** (20.1 mg, 0.033 mmol) and $Pd(OAc)_2$ (12.3 mg, 0.055 mmol) in dichloromethane were stirred at room temperature. The progress of the reaction was monitored spectrophotometrically. After completion of the reaction, the products were extracted with dichloromethane (5×30 ml) and purified with a dichloromethane–methanol mixture (50:1, v/v) to afford 88% of the product. UV (λ_{max}/nm): 393 (Soret), 596, 642. MS (MALDI TOF/TOF), *m*/*z*: 709.055 (^{105}Pd, [M+1]$^+$), 710.075 (^{106}Pd, [M+1]$^+$), 712.069 (^{108}Pd, [M+1]$^+$).

(Scheme 3).[¶] The presence of *tert*-butyl acetate groups in the latter is confirmed by the appearance of a set of new signals in the ^{1}H NMR spectrum in the regions of δ 1.0–1.5 and 4.0 ppm, which correspond to nine methyl and three methylene groups. To unblock carboxy groups, we performed acidic hydrolysis of compound **5** with 80% TFA. The decrease in the mobility of reaction product indicated that triacid **6** was formed (see Scheme 3). To obtain the homometallated complex, conjugate **6** was treated with palladium acetate in methanol. Spectrophotometric monitoring of the reaction showed that a mono-Pd complex ($\lambda_{\max}$ = 624 nm), which apparently corresponded to the incorporation of Pd into chlorin, was formed initially. With time, a hypsochromic shift of the Q_2 band to 614 nm for a di-Pd complex was observed.



Scheme 3 *Reagents and conditions*: i, $BrCH_2COOBu^t$, CH_2Cl_2, DIPEA; ii, 80% CF_3COOH; iii, $Pd(OAc)_2$, MeOH.

Thus, we have performed a direct opening of exocycle E in chlorin with cyclen and obtained metal complexes with one or two Pd^{2+} cations, which allows the suggested construction to be considered as a chelator for *d*-metals and lanthanides.

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[¶] *Dipalladium complex of chlorin–cyclen conjugate* **7**.

Step 1. *tert*-Butyl bromoacetate (0.8 ml, 0.005 mmol) was added to conjugate **2** (2.3 mg, 0.003 mmol) in dichloromethane (0.5 ml). The reaction mixture was stirred for 30 min. An increase in TLC chromatographic mobility was observed. Once the reaction was completed, the solvent was evaporated and product **5** was purified by preparative TLC, then recrystallized from a dichloromethane–hexane system. Yield, 84%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.76 (s, 1H, *meso*-H^{10}), 9.68 (s, 1H, *meso*-H^5), 8.97 (s, 1H, *meso*-H^{20}), 8.09 (t, 1H, $CH{=}CH_2$), 6.45 (dd, 1H, *trans*-3-$CH{=}CH_2$), 6.20 (dd, 1H, *cis*-3-$CH{=}CH_2$), 4.63–4.42 (m, 2H, H^{17}, H^{18}), 4.28 (s, 3H, 15-CO_2Me), 4.11 (s, 2H, 15^1-CH_2), 3.86 (m, 6H, 3CH_2 cycloalkyl), 3.83–3.70 (m, 2H, 8-CH_2Me), 3.65 (s, 3H, 17-$CH_2CH_2CO_2Me$), 3.56, 3.43, 3.39 (s, 3×3H, 2,7,12-Me), 2.94–2.78 (m, 4H, 17-$CH_2CH_2CO_2Me$), 2.45–2.06 (m, 16H, cyclo-CH_2), 1.81 (m, 3H, 18-Me), 1.70 (t, 3H, 8-CH_2Me), 1.63–1.15 (m, 27H, Bu^t), 0.80 (br.s, 1H, NH), –1.61 (br.s, 1H, NH). MS (MALDI TOF/TOF), *m/z*: 1121.521 $[M+1]^+$. UV ($\lambda_{\max}$/nm): 390 (Soret), 500, 663.

Step 2. Crystals of compound **5** (3.3 mg, 0.003 mmol) were dissolved in 80% TFA (0.2 ml). Acidic hydrolysis was carried out for 30 min with stirring. TLC showed a considerable decrease in the chromatographic mobility in comparison with the original conjugate. Once the reaction was completed, product **6** was purified by preparative TLC and recrystallized from a dichloromethane–hexane system. Yield, 86%. UV ($\lambda_{\max}$/nm): 390 (Soret), 500, 663. MS (MALDI TOF/TOF), *m/z*: 953.445 $[M+1]^+$.

Step 3. Palladium acetate (1.1 mg, 0.005 mmol) was added to a solution of conjugate **6** (2.3 mg, 0.003 mmol) in aqueous methanol (0.3 ml). The mixture was stirred in a water bath with heating to 40–45 °C for 24 h. An increase in chromatographic mobility in analytical TLC was observed. After completion of the reaction, the solvent was removed *in vacuo*. Product **7** was purified and crystallized in a dichloromethane–hexane system. Yield, 92%. UV ($\lambda_{\max}$/nm): 398 (Soret), 576, 614. MS (MALDI TOF/TOF), *m/z*: 1179.969 $[M+H_2O]^+$.