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Inverse electron demand Diels–Alder reaction as a novel method for functionalization of natural chlorins

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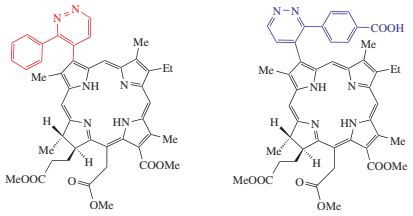
The addition of 3-aryl-1,2,4,5-tetrazines at the vinyl group of chlorin e_6 trimethyl ester affords the corresponding pyridazine–porphyrin conjugates. The study of spectral properties of the obtained compounds revealed insignificant changes in the absorbance and fluorescence spectra as compared to those of chlorin.

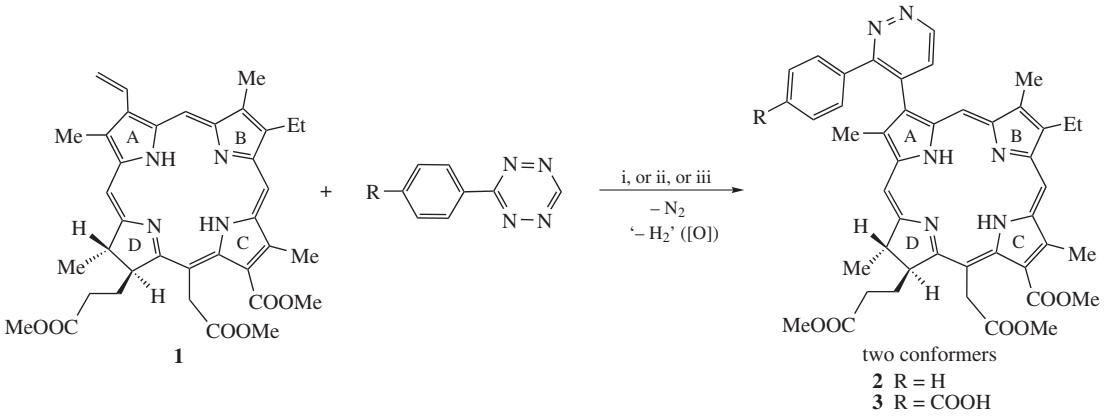


Natural chlorins are widely used as photosensitizers in photodynamic therapy.[1–4] The chemical modification of these compounds is quite an urgent problem since it allows one to affect the photophysical properties of a pigment and create conjugates with various organic molecules. It has been shown that regiodirected incorporation of such molecules considerably affects the biological activity of photosensitizers.[5–7] The majority of synthetic studies on the functionalization of chlorophyll *a* derivatives involves the 'lower' part of the macrocycle (pyrroles C and D). Nevertheless, some chemical modifications to add biologically active molecules are implemented on pyrrole A. Transformation of the vinyl group in chlorins to the 1,2-dihydroxyethyl one is widely used since the latter can be further converted to formyl or carboxy group.[8–10] Yet another promising method to modify the vinyl group involves the Ru- or Mo-catalyzed cross-metathesis with other alkenes.[11] The Heck reaction was used to add nucleoside residues by treatment of various chlorins with 2′,3′,5′-tri-*O*-acetyl-5-(chloromercurio)uridine.[12]

The use of click-chemistry approaches for minimizing side reactions that occur in operations with natural compounds became popular recently. They include azide–alkyne cycloaddition, thiol–ene addition and nucleophilic opening of strained rings.[13] A special place among click-reactions belongs to tetrazine–alkene addition also known as the 'Inverse Electron Demand Diels–Alder Reaction' (IEDDA) that does not require a catalyst.[14] In this reaction, a 1,2,4,5-tetrazine derivative acts as a 'diene' and an alkene or alkyne, as a 'dienophile'. [4+2]-Cycloaddition would proceed on C^{3,6} carbon atoms of tetrazine, and the subsequent release of nitrogen molecule and oxidation afford the pyridazine cycle (Figure S2, Online supplementary Materials). The reaction rate depends on the structures of the reactants.[15] The accessibility of a vinyl group in chlorophyll *a* derivatives makes it possible to use the tetrazine–alkene addition in a series of natural chlorins.

The purpose of this work was to study the IEDDA reaction between chlorin e_6 trimethyl ester and aryltetrazines, as well as to determine the structures of the products.

At the first stage of this study, symmetric 3,6-diphenyl-1,2,4,5-tetrazine and 3,6-bis(4-carboxyphenyl)-1,2,4,5-tetrazine were tested. However, their reactions with chlorin e_6 trimethyl ester **1** did not occur. Since non-symmetric aryl-substituted



1

2 R = H
3 R = COOH

Scheme 1 *Reagents and conditions*: i, toluene, reflux, 48 h, 73% (for **2**); ii, CH_2Cl_2, room temperature, 72 h, 80% (for **2**); iii, DMF, room temperature, 8 h, 94% (for **2**), 93% (for **3**).

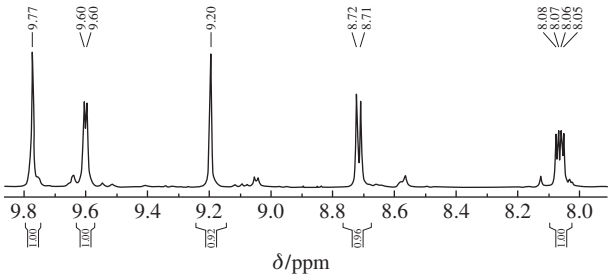


Figure 1 A fragment of the ^{1}H NMR spectrum of compound **2**.

Table 1 Data for a fragment of the NMR spectra of compound **2**.

^{1}H	δ/ppm		^{13}C	δ/ppm	
	Conformer 1	Conformer 2		Conformer 1	Conformer 2
H-5	9.20 (s)	9.20 (s)	C^5	99.4	99.4
H-10	9.77 (s)	9.77 (s)	C^{10}	102.1	102.1
H-20	8.71 (s)	8.72 (s)	C^{20}	94.1	94.2
H-6′	8.06 (d, *J* 4.7 Hz)	8.07 (d, *J* 4.7 Hz)	$C^{6'}$	130.8	130.8
H-5′	9.60 (d, *J* 4.7 Hz)	9.60 (d, *J* 4.7 Hz)	$C^{5'}$	149.6	149.6

tetrazines are known to be much more reactive,[16] we moved to 3-phenyl-1,2,4,5-tetrazine, the solvent nature having been varied (Scheme 1). The reaction in refluxing non-polar toluene led to full conversion of chlorine **1** within 48 h, and it was accompanied by partial decomposition of the product (η = 73%). When the reaction was performed in dichloromethane, its completion required 72 h at room temperature. In polar DMF the reaction rate was essentially greater, which provided 94% yield of product **2** after 8 h contact at room temperature. Carrying out the reaction on contact with atmospheric oxygen made it possible to avoid the need of oxidizing agents to obtain the pyridazine cycle. The structure of the product was studied by NMR spectroscopy. The ^{1}H NMR spectrum showed two different sets of signals corresponding to the *meso*-proton at the 20-position of the macrocycle and also two different sets of signals assigned to H-6′ of the pyridazine ring (for numbering, see Figure S1, Online Supplementary Materials). Analysis of ^{1}H, ^{13}C and HSQC spectra revealed the presence of two conformers in 1 : 1 ratio (Figure 1, Table 1). Assignment of their signals was performed using COSY and NOESY experiments. The cross peaks in the COSY spectrum show that [4+2]-cycloaddition of tetrazine to chlorin affords a compound, in which two conformational isomers can be distinguished. The NOESY spectrum of conformer 1 contains cross peaks between H-6′ and H-5 protons as well as between 2-Me and *ortho*-protons of the phenyl substituent in pyridazine. The signals of conformer 2 were assigned on the basis of the NOESY cross-peaks between the *ortho*-protons of the phenyl group and H-5, as well as the cross-peaks between the H-6′ and 2-Me protons (Figures 2 and S9).

In this study we also performed the reaction of chlorin e_6 trimethyl ester **1** with 4-(1,2,4,5-tetrazin-3-yl)benzoic acid for

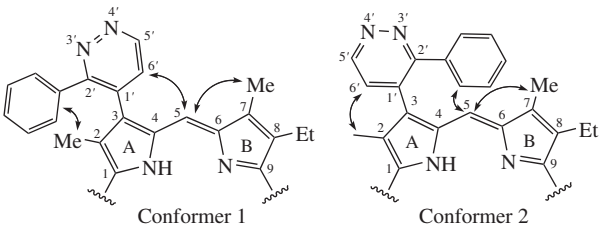


Figure 2 Fragments of the structures of conformers of compounds **2**. The arrows show the NOE effects observed in the NOESY spectrum.

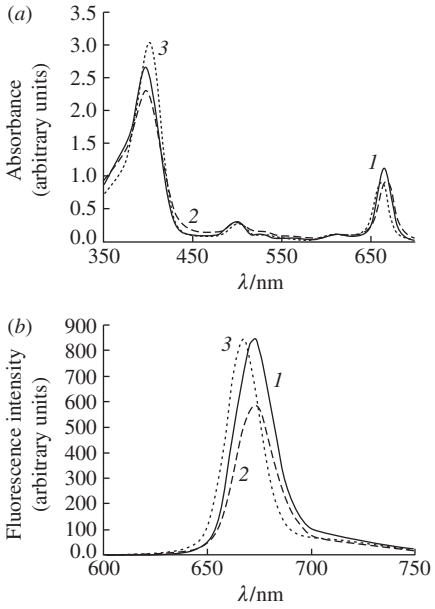


Figure 3 (*a*) Absorption and (*b*) fluorescence spectra of compounds (*1*) **2**, (*2*) **3** and (*3*) chlorin e_6.

introduction of a carboxy group into the molecule. The reactivity of this tetrazine was considerably higher. Chlorin **1** underwent full conversion within 1 h in DMF at room temperature (yield of product **3** was 93%, see Scheme 1). The ^{1}H and ^{13}C NMR spectra of compound **3** showed the presence of two conformers, similarly to the reaction described above.

Studying the photophysical properties of the compounds obtained has shown that introduction of a pyridazine moiety into the molecule displaces the long-wave absorption maximum of chlorin from 662 to 665 nm for compound **2** and to 667 nm for compound **3** (Figure 3). The chemical modification mentioned above did not change the fluorescent properties in comparison with chlorin e_6: the fluorescence quantum yields φ were 0.16 and 0.11 for the phenylpyrazine and *para*-carboxyphenylpyridazine derivatives of chlorin, respectively.

In summary, our study of the IEDDA reaction between aryl-substituted tetrazines and chlorin e_6 trimethyl ester has demonstrated that [4+2]-cycloaddition of tetrazine to the photosensitizer molecule occurs and two conformers of the formed conjugates can be distinguished, probably due to steric constraints that preclude free rotation of the pyridazine substituent. A new method for incorporation of a carboxy group to the chlorin macrocycle has also been suggested. The absorbance and fluorescence spectra of the obtained compounds are close to those of chlorin e_6.

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Online Supplementary Materials

Supplementary data associated with this article (experimental procedures, NMR and mass spectra) can be found in the online version at doi: 10.1016/j.mencom.2019.03.031.

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