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The interaction of a β-Fused isoindoline-porphyrin conjugate with nucleic acids

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A diiminoisoindolo-pyrazino unit was annulated to the β-pyrrolic position of the Zn complex of tetraphenylporphyrin, giving a macrocycle with an expanded π system in satisfying yields. The crystal structure of this derivative evidenced the formation of a hydrogen bond network. The interaction of the β-fused isoindoline porphyrin derivative with nucleotides and nucleic acid has been investigated

Nature uses porphyrins to perform essential functions that encompass the animal and plant kingdoms, going from oxygen activation and transport in animals to photosynthetic processes in plants. The possibility to mimic the distinguishing electronic properties and versatile chemistry of biological porphyrins with synthetic counterparts makes these macrocycles ideal candidates for disparate applications[1], ranging from catalysis and materials to medicine and sensing. The possibility to finely tune the porphyrin properties by synthetic modification of macrocyclic skeleton for a specific function is virtually unlimited, since numerous synthetic routes to modify both *meso*- and β-positions are already accessible[2]. In recent years, the introduction of different biomolecules[3] (carbohydrates, aminoacids, peptides, steroids and others) into the porphyrin scaffold was intensively investigated, giving a series of synthetic receptors successfully exploited for the binding of biological systems. In this context, the conjugation with N-heterocyclic compounds is particularly attractive, since these molecules are of interest for both their variegate biological relevance[4] and their versatile use as a building block for further structural modifications[5]. A typical example of this opportunity is represented by isoindoline derivatives, whose different biological activities as antibacterial[6] and antimicrobial agents[7] as well as protein inhibitors[8] are largely reported. On the other hand, properly modified isoindolines are optimal building blocks for the synthesis of various nitrogen ligands, such as benzoporphyrins[9], phthalocyanines[10] and their analogs[11] and the class of 3-bis(2-pyridylimino)isoindoline (BPI) derivatives[12]. The wide potentialities offered by the isoindoline ring prompted us to introduce this functionality into the porphyrin skeleton: the merger of these subunits indeed can produce a highly versatile porphyrin conjugate exploitable in different fields. From a synthetic point of view, we have planned to annulate the isoindole ring to a β-pyrrolic position of the porphyrin ring. Indeed this peripheral substitution allows in principle the construction of π-extended systems, which are of great interest in building electron and energy-transfer devices.

In our laboratories the preparation and characterization of β-fused porphyrin systems has just been addressed. Indeed, we recently developed an efficient synthetic route to prepare β-fused bis-porphyrin systems, where the two macrocycles are linked by a pyrazine unit, using a 2,3-diaminoporphyrin and diethyl oxalate or porphyrin-2,3-dione as reagents.[13,14] Based on this successful methodology, we obtained our target compound by constructing the isoindolo-pyrazino porphyrin system in two steps, the former being the condensation of the porphyrin-2,3-dione derivative **1** with the diaminomaleonitrile and the latter being the reaction between the dicyanopyrazinoTPP **2** with sodamide, as indicated in Scheme 1. Compound **1** was prepared by oxidizing the corresponding 2,3-diaminoporphyrin with the Dess-Martin periodinane (DMP) reagent as reported in the literature[15]. This compound was subsequently reacted with a 25-fold excess of diaminomaleonitrile (DAMN) in a refluxing CH_2Cl_2/EtOH/HOAc (5:5:1) solvent mixture, as commonly reported for the acid catalyzed condensation of o-diamines with vicinal diketones[16]. In 3 hours the reaction afforded a main greenish product that was isolated after chromatographic purification on a silica gel column, using dichloromethane as eluant. The dicyanopyrazino derivative **2** was obtained in 68% yield and was fully

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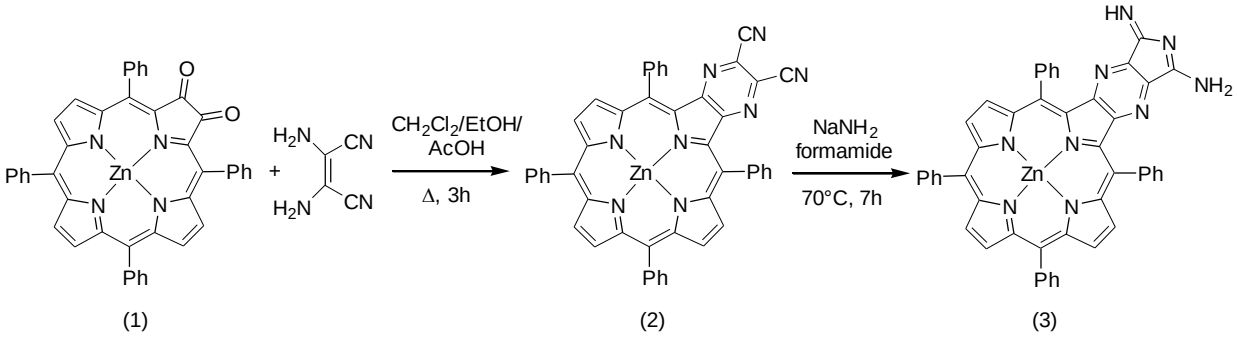

Scheme 1: Synthetic pathway for the preparation of the zinc diiminoisoindoline-porphyrin conjugate **3**

characterized by conventional spectroscopies. The UV/vis spectrum of **2** in CH_2Cl_2 features a split Soret band with two peaks at 438 and 472 nm, and a Q band centered at 599 nm. As expected, the red-shift of all the absorption bands compared with the parent ZnTPP, reveals an increase of the π-electron delocalisation degree along the porphyrin macrocycle due to the inserted pyrazine unit.

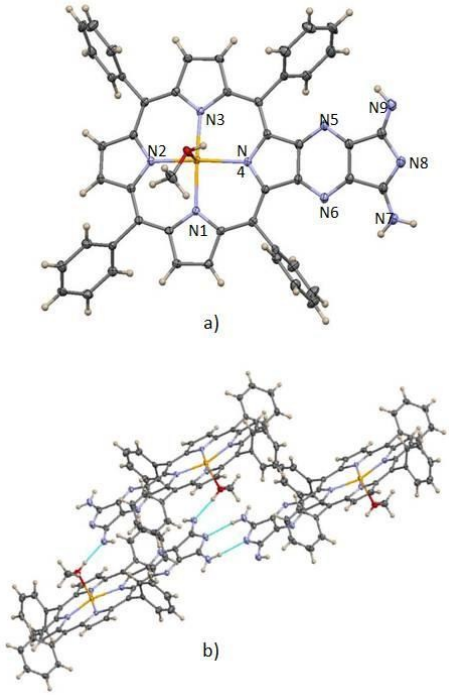

Figure 1: Molecular structure of **3** with 50% ellipsoids: a) single molecule; b) hydrogen bonding networks formed by 3 in crystal form

Porphyrin **2** was then dissolved in formamide and treated with an excess of $NaNH_2$ at 70 °C. In seven hours the desired diiminoisoindoline-porphyrin conjugate **3** was achieved in 61% yield, after precipitation with distilled water. Although the electronic spectral features for this compound were only slightly affected by the isoindoline ring formation, the ^{1}H NMR spectrum displayed broad and unresolved signals, which hampered the definitive identification of the obtained product. This line broadening can be ascribed to the aggregation equilibria of **3,** reasonably induced by the formation of intramolecular hydrogen bond networks in solution. However, we were able to identify the porphyrin-isoindoline conjugate **3** *via* single crystal X-ray crystallographic analysis[17], obtaining the molecular structures reported in Figure 1.

The Zn(II) center has square pyramidal coordination, with a methanol molecule in the axial position. Zn-N distances are in the range 2.042(2) – 2.1254(19) Å, and the Zn-O distance is 2.1415(17) Å. The longest Zn-N distance is to the pyrrole carrying the pyrazino moiety. The diiminoisoindoline exists as the tautomer in which the central isoindoline N atom is deprotonated, and one of the imino N atoms carries an extra H atom, existing as NH_2. These groups form an intermolecular hydrogen-bonded dimer in the solid, as seen in Fig. 1b, and the other imino N atom accepts a hydrogen bond from coordinated methanol. The isoindoline C_5N ring exhibits localized bonding, with the C-N distance on the NH_2 side, 1.334(3) Å, having much more double bond character than the one on the N-H side, 1.401(3) Å. Likewise, the imino C-N distances are asymmetric, with C-NH, 1.264(3) Å shorter than C-NH_2, 1.314(3) Å. The Zn atom lies 0.322 Å from the best plane of the 24-atom porphyrin core. The mean deviation of the 24 atoms from coplanarity is 0.073 Å, with the β C atoms opposite the diiminoisoindoline subsituent deviating the most. The conformation of the 24- ring does not closely resemble any of the ideal forms. Due to the presence of different nitrogen-containing groups into the isoindoline structure, endowed with donor or acceptor abilities of H-bonds, it was thought of interest to investigate the potential interaction of this porphyrin conjugate with selected biomolecules. For a long time porphyrinoid–nucleic acids complexes have been widely studied to develop a novel range of supramolecular constructs for applications in medicinal chemistry,[18] but to date, there are very few artificial receptors for nucleotides that possess the capability to strongly discriminate between the five nucleotides. [19]

The introduction of the isoindoline group into the porphyrin macrocycle allows us to exploit the ability of this nitrogen-rich group to form H-bonds. In particular the combination of amino and amide groups able to act as donor or acceptor of H-bonds make this porphyrin very suitable to interact with nucleotides. However the development of receptors based on hydrogen bonds in water solution results in a lack of significant nucleotide selectivity due to the competition of water media.

The porphyrin-isoindoline conjugate **3** exhibits low solubility in water; however using a DMSO:water (4:1) solution it is

possible to avoid intermolecular association within the concentration range 0.01–5x10^{-4} M and to analyze the affinity with nucleic acids. We tested the titled porphyrin with all five nucleotides (GMP, AMP, CMP, TMP and UMP) by UV/Vis titration (see ESI Figure S5). In UV/Vis spectra the Soret band of porphyrin **3** exhibits small, but significant red shifts (Δλ ~ 5nm) and a pronounced hypochromic effect (H ~ 20%), as a result of the addition of increasing amounts of nucleotides, indicating that this porphyrin interacts with all five nucleotides (Table 1). The spectroscopic properties of porphyrin **3** with the different nucleotides reveal minor differences. The absence of any significant Watson-Crick type of hydrogen bonding selectivity can be explained by the high number of possible combinations of donor/acceptor H-bonds displayed by both nucleotides and the diiminoisoindoline porphyrin derivative. This was also highlighted by the determination of binding constants (K_b) for all five porphyrin-nucleotide complexes (Table 1). The obtained K_b values was found to be in the range 10^5 M^{-1} in agreement with values reported in literature.[20]

Table 1. The % Hypochromicity (% H), Soret shift (Δλ) and binding constant (K_b) for **3**-nucleotides complexes

	% H	Δλ nm	K_b M^{-1}
AMP	20	5	2.2×10^5
GMP	18	3	2.4×10^5
CMP	23	6	2.4×10^5
TMP	19	5	2.2×10^5
UMP	21	6	2.5×10^5

Reporting the absorption Soret values at 444 nm versus the concentration of added nucleotides, a 1:1 porphyrin:nucleotide complex stoichiometry for all compounds analyzed is obtained. These results were highlighted by the presence of one break point at 1:1 porphyrin:nucleotide ratio for each titration (ESI, Figure S5). After pointing out the ability of this conjugate to interact weakly with nucleotides, in spite of the competition of the solvent used (water and DMSO), we decide to investigate its affinity for double stranded polynucleotides.

The non-covalent interaction between porphyrins and double-stranded DNA has been widely investigated since 1945, with the pioneering work performed by Gupta and coworkers.[21] This led to a large amount of work appearing in the literature on porphyrin–DNA non-covalent complexes.[22] The most significant characteristic promoting the interest of the scientific community to study the binding of porphyrinoids with DNA, is the presence of heteroaromatic chromophores having the ability to intercalate with DNA bases by π–π interactions, inhibiting the transcription process and acting as photosensitizers with high efficiency in PDT.[23] Among the long list of polynucleotides available we choose to investigate the interaction of **3** with poly(dG-dC). The GC-rich repeats are localized both in coding and non-coding regions of genes[24] and are predominantly involved in fragile X neurodisorder.[24,25] Further, the expansion of these repeats differ, depending on the location of the repeat tract in the gene, and the consequences range from reduction of transcription initiation to protein toxicity.[25]

By UV/vis (see ESI, Figure S6) and CD (Figure 2) spectroscopies, no ~~binding~~ interactions have been detected ~~in the presence of the~~ between **3** and poly(dG-dC) double helix. It might find explanation on the absence of interactions in two structural aspects of **3**: *i)* the presence of axial ligand that hinder the spontaneous intercalation amid the basis, and *ii)* the lack of positive charges that avoid electrostatic interactions. However it is possible to force the inclusion of porphyrins inside the polynucleotides inducing the formation of double helix in the presence of porphyrins.[26] Also in ~~this~~ our case after increasing the temperature of the porphyrin/poly(dG-dC) solution up to 90 °C, (which is the melting temperature of poly(dG-dC)) and then cooling down fast up to room temperature, an induced CD band appears in the porphyrin absorption region (Figure 2) and red shift (8 nm) in the Soret band is detected (ESI, Figure S6). The CD spectrum of poly(dG-dC) in the presence of **3** after temperature variation experiment, does not show any significant changes, indicating that the induced interaction does not perturb the double helix structure of poly(dG-dC) even after 1 h (Figure 2 and ESI, Figure S7).

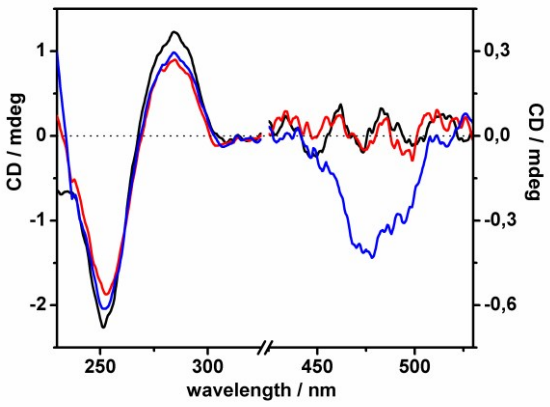

Figure 2: CD spectra of poly(dG-dC) 10 μM solution in cacodilate buffer 1mM (black curve) in the presence of **3** 4μM (red curve), after increasing the temperature to 90°C and cooling down fast to 25°C (blue curve).

This experiment indicates that it is possible to achieve intercalation inside the poly(dG-dC) double helix of the diiminoisoindoline-porphyrin conjugate, as pointed out by the negative ICD signal typical for intercalative binding,[27] inducing the formation of double helix of polynucleotide in the presence of **3**.

We here reported a simple and efficient synthetic route for the introduction of a diiminoisoindoline group into β-pyrrolic position of the porphyrin macrocycle. The X-ray crystallographic analysis elucidated the structure of this new porphyrin derivative, evidencing the formation of H-bond networks in the solid state. The potential interaction *via* hydrogen bonds with nucleotides was also investigated. In these studies it is demonstrated that although **3** exhibits low solubility in water, it is able to interact with nucleotides

forming a 1:1 complexes with all nucleotides. In particular, we are able to promote inclusion of the titled porphyrin into poly(dG-dC) double helix by inducing the fast formation of double helix. Together with this supramolecular aspect, we believe that this novel porphyrin derivative is of significant interest in the porphyrinoid field, being a versatile compound to use as a starting substrate for following laterally expanded porphyrinoid systems and porphyrin-BPI bimetallic complexes. These topics are currently ongoing in our laboratories and the results will be reported in due course.

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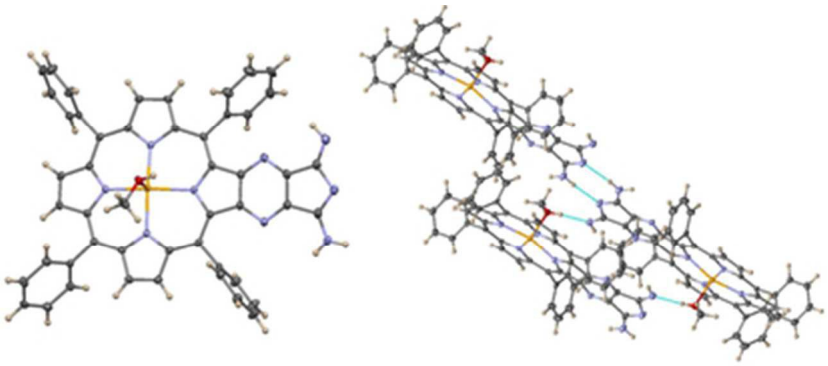
The intercalation of a diiminoisoindoline-porphyrin into the poly(dG-dC) double helix occurs by inducing the fast formation of helix with temperature.



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