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Synthesis of DNA Conjugates with Metalated Tetracationic Porphyrins by Postsynthetic Cycloadditions

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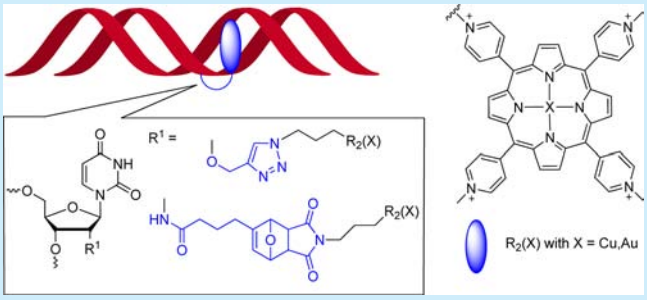
Supporting Information

ABSTRACT: Tetracationic porphyrins of the TMPP (*meso*-tetra-(4-*N*-methylpyridyl)porphyrin) type, metalated with Cu(II) or with Au(III), were conjugated covalently to oligonucleotides. The Cu(I)-catalyzed cycloaddition (between an azide and an ethynyl group) and the Diels–Alder cycloaddition (between a furan and a maleimide functionality) were successfully applied as two alternative postsynthetic methods to modify the 2′-position of an internal uridine. Melting temperatures and UV/vis absorption properties were compared. CD measurements indicated that the type of conjugation chemistry determines the grade of intercalation of the attached and positively charged porphyrins.



Over the last 10 years, DNA became an increasingly attractive basis for supramolecular architectures to arrange covalently attached chromophores and fluorophores in a precise fashion on the nanoscale.[1] Among the various organic chromophores, porphyrins and their derivatives have significant potential since they have unique properties which are used, e.g., construction of artificial light-harvesting complexes[2] and use for photodynamic therapy.[3] Noncovalently formed helical porphyrin nanoassemblies were formed using single-stranded DNA templates.[4] The synthesis of covalent DNA–porphyrin conjugates has been achieved in four different ways: (i) porphyrins linked as DNA base modifications (mainly to the 5-position of 2′-deoxyuridine) via ethynyl bridges allow construction of DNA zippers for efficient energy transfer;[5] (ii) porphyrins covalently attached as 5′-caps improve base pair fidelity[6] and are applied as chiroptical probes;[7] (iii) porphyrins attached to the phosphodiester backbone of DNA[8] have been used to form DNA nanoassemblies;[9] (iv) porphyrins covalently attached to the 2′-position stabilize the formation of duplexes and triplexes.[10]

Tetracationic and therefore water-soluble porphyrins belong to the most thoroughly studied noncovalent DNA binders; there are many early reports[11,12] and a few X-ray structures available.[13] The type of interaction (groove binding or intercalation) with DNA depends on the number of positive charges on the porphyrin.[14] In particular, the tetracationic *meso*-tetra-(4-*N*-methylpyridyl)porphyrin (TMPP) is well-known as a potent DNA intercalator.[4−6,14−16] Copper ions in the center of TMPPs flatten the porphyrin chromophore and thereby enhance the binding affinity toward double-stranded nucleic acids.[15] The intercalative binding of tetracationic porphyrins also improves their ability as photosensitizers.[16]

Covalent TMPP–DNA conjugates would represent not only interesting candidates for sequence-specific photoinducable DNA or RNA cleavage in vivo but also promising structures for nanosized architectures and molecular electronics with DNA. The tetracationic porphyrin of the TMPP type represents a special challenge for nucleic acid conjugation chemistry. Since the synthesis of a corresponding phosphoramidite as DNA building block seemed to be very unreasonable with respect to the tetracationic character and the expected poor solubility; we chose postsynthetic approaches to modify the 2′-position of uridine as part of presynthesized oligonucleotides. Herein, we present two alternative postsynthetic protocols to covalently link the TMPP-type porphyrins metalated with Cu(II) or Au(III) to DNA, and compare them by their resulting UV/vis absorption and CD spectroscopy properties.

The first method applies the copper(I)-catalyzed cycloaddition of the tetracationic porphyrin **1** bearing an azide group with the 2′-propargylated uridine as part of the oligonucleotide precursor **DNA1** (Scheme 1). This postsynthetic strategy at the 2′-position was established by us for a variety of different chromophores and works with 60–70% yield.[17] The corresponding DNA building block is commercially available. The second method is based on a Diels–Alder reaction between the porphyrin **2** modified with a maleimide function and a uridine in the oligonucleotide precursor **DNA3** which carries a furan moiety in the 2′-position. The corresponding uridine phosphoramidite as DNA building block was synthesized according to literature procedures (see the Supporting Information).[18] This postsynthetic Diels–Alder methodology was initially established by the group of Madder using cytidine and adenosine derivatives and a different linkage to the furan moiety.[18]

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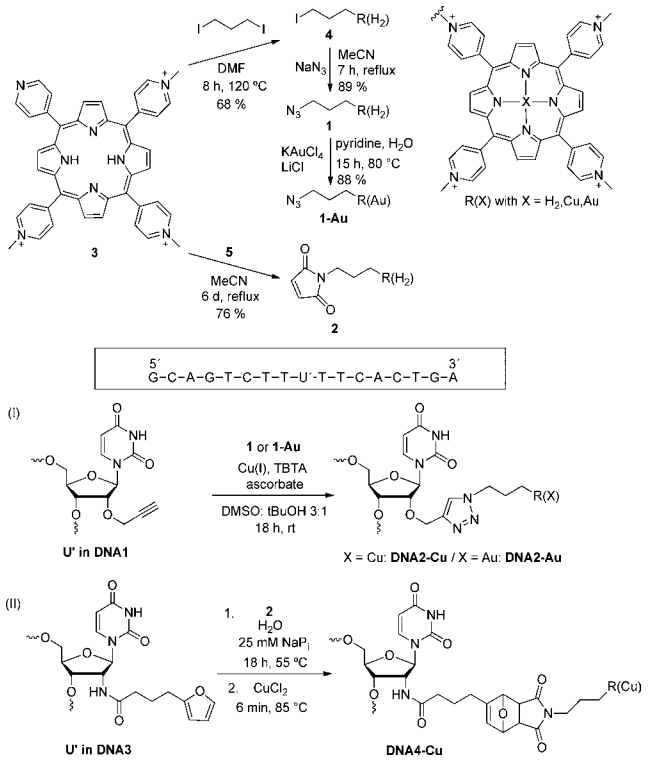
Scheme 1. Synthesis of the Tetracationic Porphyrins 1 and 2 and Postsynthetic Modification of Oligonucleotides by Two Alternatives (I and II)



The synthesis of both porphyrins, **1** and **2**, starts from *meso*-(4-pyridyl)-tri(4-*N*-methylpyridyl)porphyrin (**3**) that was obtained by condensation of pyrrole with pyridine-4-carbaldehyde to *meso*-tetra(4-pyridyl)porphyrin[19] and subsequent 3-fold methylation performed according to literature (see the Supporting Information).[20] The azidopropyl linker of porphyrin **1** was attached by alkylation of the remaining pyridyl group of porphyrin **3** with 1,3-diiodopropane (68% yield) and subsequent nucleophilic substitution of the fully alkylated porphyrin **4** with NaN_3 in MeCN (89% yield). The maleimide functionalized linker of porphyrin **2** was introduced by alkylation of porphyrin **3** with 1-(3-iodopropyl)-1*H*-pyrrol-2,5-dione (**5**)[21] (76% yield). Metalation of porphyrins was achieved according to literature procedures.[22,23]

The postsynthetic modifications were attempted with an arbitrarily chosen sequence carrying the corresponding, bioorthogonally reacting 2′-group at a uridine in the middle. The "click"-type cycloaddition of **DNA1** with the metal-free porphyrin **1** using our published procedure[17] gave directly the conjugate **DNA2−Cu** as identified by MALDI-TOF MS. Obviously, the Cu(II) ion was inserted into the porphyrin moiety during the postsynthetic modification procedure. Thus, for the synthesis of **DNA2−Au** it was necessary to incorporate Au(III) into the porphyrin prior to the postsynthetic modification. The corresponding building block **1−Au** was obtained by treatment of **1** with an aqueous solution of $KAuCl_4$, LiCl, and pyridine at elevated temperature.[22] The metal insertion was followed by UV/vis absorption spectroscopy (shift of the Soret band from 423 to 406 nm, data not shown) and by mass spectrometry.

The Diels−Alder-type postsynthetic modification is a metal-free methodology and thus more flexible with respect to metal insertion into the porphyrin. The reaction of **DNA3** with porphyrin **2** gave **DNA4** in 32% yield and was subsequently treated with an aqueous solution of $CuCl_2$ to yield **DNA4-Cu**.[24] Excess metal ion salts were removed by desalting with oligonucleotide purification cartridges. Alternatively, porphyrin **2** can first be metalated by $CuCl_2$[24] and subsequently attached to **DNA3**. That means that the order of metal insertion and postsynthetic DNA modification does not play any role in this postsynthetic route.

The modified single strands were annealed with counter-strands that were complementary except the position X opposite to the 2′-modified U in **DNA2−Cu/Au** and **DNA4−Cu** (Table 1). The resulting double strands were characterized by their melting temperatures (T_m) that were measured at 260 nm. The reference T_m of a completely unmodified and complementary duplex is 59.5 °C. The T_m values of the two sets of "click"-type modified double strands (**DNA2−Cu** and **DNA2−Au**) show the highest value with X = A. Compared to the unmodified duplex, especially **DNA2−Cu** with X = A exhibit a strong stabilization (+2.6 °C) by the attached porphyrin. All "mismatched" cases (X = C, G or T) showed T_m values that are 3.0−8.0 °C lower. Obviously, there is a significant preference of the 2′-modified U for A in the opposite position which indicates a matching base pairing situation between both strands that persists also in the presence of the porphyrin as 2′-modification. This observation was not made with the duplexes of **DNA4−Cu** that were postsynthetically modified by the Diels−Alder reaction (Table 1). The corresponding T_m values do not exhibit any preference for X = A since the differences for X = C and G are rather small (0.2−2.7C) and the double strand of **DNA4−Cu** with X = T opposite to the modified U ("mismatch") is 2 °C more stable than the duplex with X = A ("match").

Table 1. Characteristic UV/vis Absorption Data (Soret Band and Q band) and Melting Temperatures (T_m) of DNA2-Cu, DNA2-Au, and DNA4-Cu[a]

DNA sample		T_m (°C)	Soret (nm)	Q (nm)
DNA2-Cu	ss		434	551
	A	62.1	443	558
	C	58.4	432	552
	G	54.1	436	552
	T	59.1	430	549
DNA2-Au	ss		411	527
	A	58.6	414	531
	C	55.1	412	528
	G	54.8	410	528
	T	54.7	412	528
DNA4-Cu	ss		432	551
	A	57.1	431	548
	C	56.9	431	548
	G	54.4	432	549
	T	59.1	430	549

[a]Single-stranded (ss) DNA2 and DNA4 were annealed with oligonucleotides of the sequence 5′-T-C-A-G-T-G-A-A-X-A-A-G-A-C-T-G-C-3′ varying in the position X = A, C, G, T.

The Soret and Q bands of the porphyrins were measured by UV/vis absorption spectroscopy to compare the optical result of the two different postsynthetic modification strategies (Figure 1). This comparison between the "click"-type modified single and double strands of **DNA2−Cu/Au**, and the Diels−

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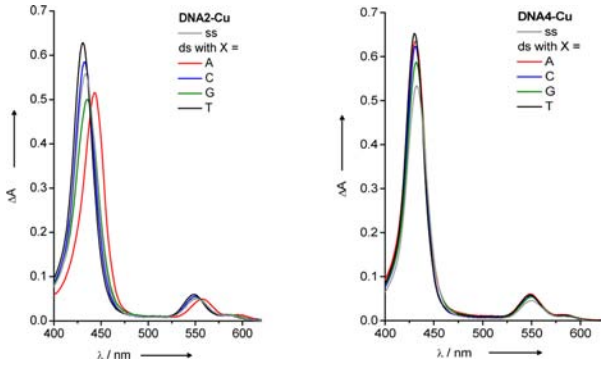

Figure 1. UV/vis absorption spectra of **DNA2−Cu** (left) and **DNA4−Cu** (right), each single-stranded (ss) and double-stranded (ds) with X = A, C, G and T, 2.5 μM in 10 mM sodium phosphate buffer, 250 mM NaCl, pH = 7.0. For **DNA2−Au**, see the Supporting Information.

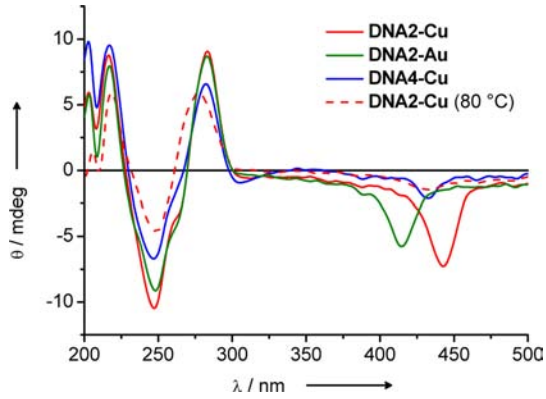

Figure 2. CD spectra of **DNA2−Cu** (20 and 80 °C), **DNA2−Au**, and **DNA4−Cu**, each at 20 °C, 2.5 μM in 10 mM sodium phosphate buffer, 250 mM NaCl, pH = 7.0.

Alder adduct **DNA4−Cu** revealed that the Soret and Q-band is shifted bathochromically only in case of the first type of modification. This was most significantly be seen with **DNA2−Cu** and the counterstrand with X = A (match); in this case, the Soret band is shifted by +9 nm. The shift is less pronounced (+3 nm) but still observable in case of **DNA2−Au**. It is important to mention here that titration experiments with arbitrarily chosen sequence double-stranded DNA into a solution of TMPP-Cu and TMPP-Au (see the Supporting Information) showed similar bathochromic shifts. Hence we assign these shifts to intercalation of the tetracationic metal ion−porphyrins into the DNA base stack, as similarly described in literature for the noncovalent type of binding of these metalated chromophores.[14−16] Interestingly, such bathochromic shifts of the Soret band were not observed with the Diels−Alder product **DNA4−Cu**. Within the experimental error, the Soret bands of the corresponding single and double strands are the same. We conclude that the bulkiness of the tricyclic linker that is formed by the cycloaddition between furan and maleimide sterically hinders the intercalation of the attached metalated pophyrins.

CD spectra were measured for **DNA2−Cu**, **DNA2−Au**, and **DNA4−Cu**. Besides the typical signals for the B-like DNA duplex between 220 and 300 nm, there are CD signals observable at 442 nm (**DNA2−Cu**), 433 nm (**DNA4−Cu**), and 415 nm (**DNA2−Au**). Within the experimental error, these values are identical to the absorption maxima of the corresponding Soret bands (see Table 1). But more importantly, these negative peaks are characteristic for the mode of porphyrin interaction and have been assigned to intercalation.[25] Interestingly, the signal intensity is highest in case of **DNA2−Cu**, and slightly lower in case of **DNA2−Au**. Assuming that no axial ligands are bound in both cases,[26] the structure of the porphyrins in both **DNA2−Au** and **DNA2−Cu** seems to be flat enough to intercalate efficiently into the DNA base stack. The slightly diminished CD signal intensity for **DNA2−Au** at 415 nm could possibly indicate that the structure of the porphyrin in **DNA2−Au** is not as flat as that of the porphyrin in **DNA2−Cu** and hence is not intercalated equally well.[15]

A remarkable difference, however, is observed with the Diels−Alder product **DNA4−Cu**. Only a weak peak is observed in the CD spectrum that indicates a less pronounced intercalation. The intensity is comparable to that of **DNA2−Cu** at 80 °C. At this temperature above the T_m of **DNA2−Cu** (see Table 1) intercalation becomes impossible due to the loss of the double-helical secondary structure. Presumably, the bulkiness of the triyclic part of the linker in **DNA4−Cu** prevents efficient intercalation even of this flat porphyrin. Hence the conjugation chemistry plays a key role for the optical properties of the ligated chromophores.

In conclusion, we worked out two different postsynthetic protocols to link tetracationic porphyrins of the TMPP type covalently to oligonucleotides. The two alternatives rely on the Cu(I)-catalyzed cycloaddition between an azide and an ethynyl group and the Diels−Alder cycloaddition between a furan and a maleimide functionality. Both methodologies were successfully applied to attach metalated porphyrins. We expect that these procedures can also be used for other metalated cationic as well as uncharged porphyrins. A special focus must be put, however, on the design of the linker between oligonucleotide and porphyrin. The comparison of the melting temperatures and the optical properties indicated that the conjugation chemistry influences the binding of the attached tetracationic porphyrins. Intercalation represents one of the unique intrinsic properties of porphyrins of the TMPP type, especially of those metalated with Cu(II), and was most efficiently obtained with the triazolyl conjugation. In contrast, the tricyclic part of the linker formed by the Diels−Alder cycloaddition seems to hinder efficient intercalation. The synthesis of TMPP−DNA conjugates as presented herein is important for the development of sequence-specific photoinducable DNA or RNA cleavage probes in vivo and of promising structures for nanosized architectures and molecular electronics with DNA.

■ ASSOCIATED CONTENT

Supporting Information

Synthesis of the used porphyrins and the furan-modified nucleoside, postsynthetic modification procedures, as well as the optical spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

All authors have contributed equally to this manuscript.

Notes

The authors declare no competing financial interest.

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