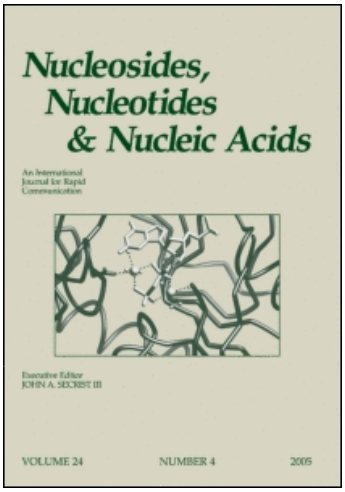

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Porphyrin-DNA: A Supramolecular Scaffold for Functional Molecules on the Nanometre Scale

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PORPHYRIN-DNA: A SUPRAMOLECULAR SCAFFOLD FOR FUNCTIONAL MOLECULES ON THE NANOMETRE SCALE

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□ *We are pursuing the aim to use DNA as a supramolecular scaffold for the creation of electronically functional molecules on the nanometre scale. Here, we give a review on our results on porphyrin modified nucleotides used for this purpose. A general synthetic route to porphyrin-nucleotides has been devised, and the building blocks can be incorporated into oligonucleotides using standard solid phase synthesis methods. Up to 11 porphyrins were incorporated into DNA, reaching a length of approximately 4 nm in the array. The spectroscopic data are consistent with a porphyrin induced secondary structure stabilisation in the single strands.*

Keywords Porphyrin nucleotides; supramolecular scaffold; nanomaterials

INTRODUCTION

Aiming at using DNA as a supramolecular scaffold, modified oligonucleotides have recently become attractive to produce nanoscaled entities and show increasing importance in nanobiotechnology.[1,2] The double stranded DNA (dsDNA) has so far mainly been used because of its high selectivity in recognition through base-pairing, and to specifically connect nano particles, in DNA chip technology and nanolithography, to create nanomechanical devices or to construct protein arrays and nanowires.[3–6] Only few reports exist where the nucleobases themselves have been

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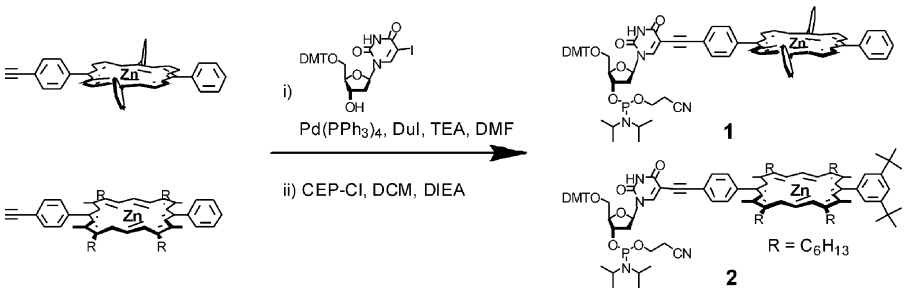
substituted to create a functional DNA,[7] for example, using "locked" DNA building blocks (LNA) substituted with methoxy or piperazino groups,[8] poly-aldehyde modified DNA for the deposition of Ag(0),[9] and direct attachment of pyrenes to the nucleobases to construct a self-assembled helical array.[10]

In this respect, we are in the course of exploring DNA to connect multiple porphyrins which are attached directly to the nucleobases through rigid phenyl acetylene spacers.[11] Upon hybridization with the complementary strand, the porphyrin units should be placed in a predetermined three-dimensional orientation, more precisely in the major groove of the double helix, giving access to new multiporphyrin arrays on the nanometre scale. So far, mainly non-covalent interactions of porphyrins with DNA, i.e., groove binding and intercalation,[12–15] single-porphyrin modifications[16,17] or post-synthetically derivatised DNA[18,19] were studied. Our system would offer a complementary template to peptidic arrays.[20,21]

DISCUSSION

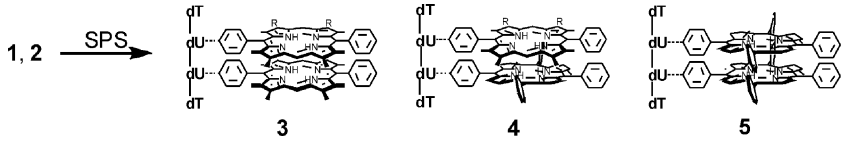
We have devised a general synthetic route to porphyrin substituted uridine and 2′-deoxyuridine using *Sonogashira* coupling with acetylene substituted porphyrins (Scheme 1).[11] Both diphenyl and tetraphenyl porphyrins, as free base or zinc metallated, can be attached to the nucleobase. Selective TBDMS or DMT protection of the deoxyribose does not affect the coupling reaction. The porphyrins themselves can be substituted with a variety of functional groups, for example esters, phenyl groups or hexyl chains for improved solubility in organic solvents, or carboxylic acids to render the conjugates soluble in aqueous solutions. However, we found that the use of tetraphenyl porphyrin (TPP) and the hexyl-di-*tert*-butyl diphenyl porphyrin (DPP), in the zinc metallated form, gave the best results in terms of yields and solubility. Thus, the phosphoramidite building blocks TPP **1** and DPP **2** are now mainly used in our studies.



SCHEME 1 General synthetic route to the porphyrin-nucleotide phosphoramidite building blocks.

In a first study, the building blocks **1** and **2** were dimerised to the homo- and hetero-porphyrin dinucleotides.[22] The syntheses were performed either in solution or on solid phase in order to compare the reactivity of the phosphoramidite building blocks under both conditions. The absorbance properties revealed electronic interactions in the dimers, which are strongly dependent on the nature of the porphyrin. The DPP containing dimers showed significant differences between the calculated and the measured UV-vis spectra, whereas in the TPP dimer hardly any difference was observed. Formation of the duplex with the corresponding diadenosine changed the electronic interactions between the chromophores in the heteroporphyrin dimer, shown by a blue shift of the absorbance. The low solubility of the DPP dimer in pure chloroform prevented formation of the duplex due to the necessity to add about 10% of methanol. The dimerisation was also partially detectable using MALDI-TOF mass spectrometry.

Since the yields of the dinucleotide synthesis in solution were far from satisfactory, we further elaborated the use of solid phase oligonucleotide synthesis with our building blocks to obtain the tetranucleotide diporphyrin arrays **3–5** (Scheme 2).[23] This method now allows the synthesis of (chiral) homo- and heteroporphyrinic arrays in the free base and zinc metallated form, where the composition and thus the physical properties of the array can be modulated simply by reprogramming the DNA-synthesizer. The UV-vis spectra of the arrays are reproducible by a superposition of the absorbance spectra of the individual porphyrins, indicating an undisturbed electronic ground state of the porphyrins in the arrays. The same is true for the steady state emission spectra of the homoporphyrinic arrays **3** and **5**. In the mixed porphyrin array **4**, large differences in the excited state properties compared to an equimolar mixture of the building blocks are observed: The emission of the diphenyl porphyrin moiety is quenched to a large extent, and the overall emission is dominated by the tetraphenyl porphyrin. First experiments using time resolved emission spectroscopy seem to show that an efficient energy transfer from the DPP moiety (Donor) to the TPP part (Acceptor) occurs; however, further measurements to confirm this finding need to be done, and these are currently in progress. Addition of a complementary tetra-adenosine did not alter any of the spectroscopic properties, neither in chloroform nor in acetonitrile solutions. Therefore, it can be concluded that no duplex is formed, which is corroborated by



SCHEME 2 Solid phase synthesis of homo- and hetero-porphyrin dimers.

^{1}H NMR spectroscopy. Even though a strong interaction between the base-pairs would have been expected to occur in organic solvents, based on the reported A-T and G-C pairing in $CHCl_3$ and DCM with K_a values in the order of 10^2 to 10^3 M^{-1},[24] this is obviously not the case in the tetra-nucleotides, even when using the natural nucleotides.

Further exploration on the use of longer oligonucleotides (ODNs) to create multiporphyrin arrays is now our major interest. Both building blocks **1** and **2** can be incorporated into longer ODN's; however, the system comprising the porphyrin **2** is not yet fully investigated. As for **1**, this porphyrin-nucleotide was incorporated site specifically into oligo-deoxynucleotides **6–9** for first investigations (Figure 1). The successful incorporation of eleven porphyrins in a row into sequence **9** shows that there seems to be virtually no synthetic limitation in the amount of modifications per DNA strand. The array in **9** corresponds to a full helical turn and an approximate length of 4 nm according to the standard DNA model. We have performed spectroscopic studies (absorption, emission and CD spectroscopy) to determine the influence of the porphyrin substitution on structure and stability of the modified DNA, and to see whether the electronic properties of the porphyrins are altered upon placement in the major-groove of the dsDNA. On average, the porphyrins destabilise the duplex by approximately −6°C

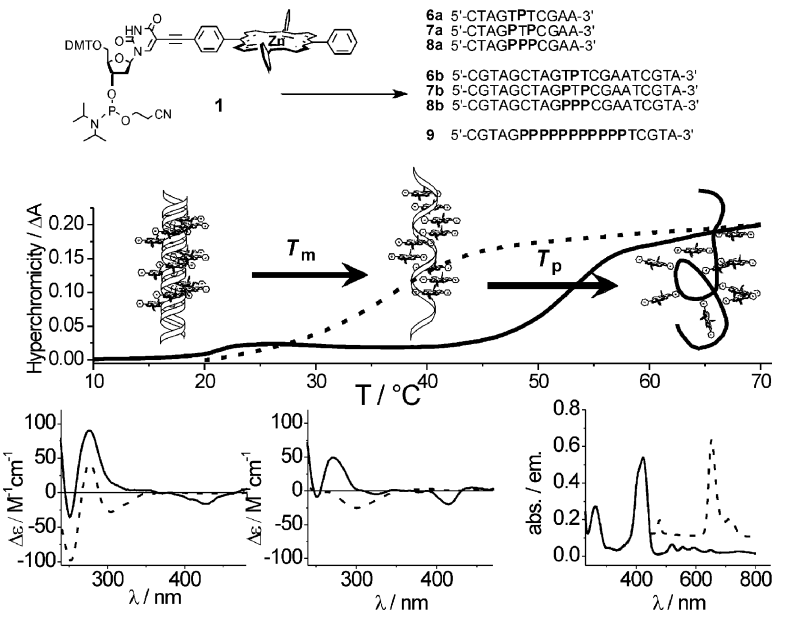


FIGURE 1 Top: Porphyrin-modified DNA sequences. Bottom: Schematic representation of the two-stage melting process in the porphyrin-DNA arrays with the melting curve, CD-spectra at 15°C and at 40°C for **9** (dotted line: unmodified 11-mer DNA). Bottom right: examples of UV-vis (solid) and luminescence (dotted) spectra for **8a**.

per porphyrin modification. From the spectroscopic investigations on the porphyrin-DNA, we come to the conclusion that the covalent attachment of porphyrins to DNA leads to an induced secondary structure stabilisation in the single stranded DNA (ssDNA) which is stable up to ~50°C, as can be seen by the thermal denaturing measurements. According to CD spectroscopy, the structure in the single strand is comparable to B-type DNA but in a more "relaxed" (extended) form. Both ss- and dsDNA porphyrin arrays show similar electronic communication between the chromophores at ambient temperature due to the enforced stacking along the DNA backbone. Depending on the length of the ODN and the amount of porphyrins, a two-stage melting process can be observed with $T_m \neq T_p$ (Figure 1), where T_m is the melting temperature of the duplex, and T_p is the unwinding temperature of the ssDNA porphyrin system.

In summary, we have shown that the backbone of natural DNA can be used to sequence specifically assemble multiporphyrin arrays and to place the units in a predetermined three-dimensional arrangement in the major groove of the dsDNA, leading to new stacked arrays on the nanometre scale. The unusually stable secondary structure of the single strands also means that in these systems the complementary strand is actually not necessary to obtain a stacked porphyrin array. It can be assumed that DNA will lead to new functional molecules on the nanometre scale when substituted with a diversity of electronically active molecules such as porphyrins, transition metal complexes or amino acid side-chain derived groups, which we anticipate in future.

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