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Porphyrin-Substituted Dinucleotides: Synthesis and Spectroscopy

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Abstract: Deoxyuridine, which is substituted with either 5,15-diphenyl porphyrin (DPP) or 5,10,15,20-tetraphenyl porphyrin (TPP) at the 5-position *via* an alkynyl linker, was dimerised to the homo- and hetero-porphyrin dinucleotide. The synthesis was 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 reveal electronic interactions in the dimers that are strongly dependent on the nature of the porphyrin. The DPP-containing dimers show significant differences between the calculated and the measured UV-Vis spectra, whereas in the TPP dimer hardly any difference is observed. Formation of the duplex with the corresponding diadenosine changes the electronic interactions between the chromophores in the heteroporphyrin dimer, as 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 is detectable using MALDI-TOF mass spectrometry for all dinucleotides.

Keywords: Dinucleotide · MALDI-TOF MS · Modified nucleotide · Porphyrin · Spectroscopy

Introduction

The use of substituted nucleotides to modify DNA has attracted considerable interest in the past years. Amino acid side chain derived substituents, chromophores for FRET analysis and even transition metal complexes have successfully been incorporated into oligo-deoxy nucleotides (ODNs), either *via* standard solid support synthesis or using PCR [1]. Aiming at using DNA as a supramolecular scaffold, DNA has recently also been used to create new nanoscale architectures [2]. However, modified nucleotides have so far not been used to connect multiple photophysically or electrochemically active molecules through the phosphodiester linkage. Upon formation of the duplex with the complementary strand, the units would be placed in a predetermined three-dimensional arrangement irrespective of the substitution on the nucleobase. As part of our research evaluating the use of DNA as a supramolecular scaffold to create multiporphyrin arrays, we have synthesised dinucleotides from the corresponding phosphoramidite building blocks, and investigated their basic electronic properties as well as duplex forming abilities.

We have recently described a general synthetic route to porphyrin-substituted deoxyuridine *via* Sonogashira coupling of alkynyl-porphyrins with iodo-deoxyuridine [3]. This way, we can introduce porphyrins with variable substitution pattern (diphenyl *vs*. tetraphenyl porphyrin) and metallation state (free base or zinc metallated). The 5'-TBDMS protected phosphoramidite building block **1** (Scheme 1) was readily obtained from the precursor dU^{ZnTPP} using standard phosphorylation procedures. We found that the phosphorus is very sensitive to oxidation due to the presence of the porphyrin as a photosensitiser [4], thus standard inert atmosphere techniques had to be applied throughout the manipulations. For the solution-phase synthesis of the dimers, the phosphoramidite **1** was used without further purification, and transferred *via* Teflon canula into a DCM solution of the corresponding DPP or TPP substituted 3'-TBDMS porphyrinyl deoxyuridines, respectively. These solutions contained 1.5 equivalents of N-(phenyl)imidazolium triflate as activator [5] for the coupling reaction. After 2 h of reaction time, the phosphonate was oxidised by addition of ^{t}BuOOH and stirring for 15 min. Then the solution was evaporated, and the product isolated by successive chromatography using preparative TLC (DCM/MeOH 5:1) and sephadex LH20 (DCM/MeOH 10:1). The purification of the desired dinucleotides proved to be tedious, because repetitive preparative TLC was necessary to remove some side products that tended to smear over the plate. The β-cyanoethoxy group was subsequently removed by treatment of the purified dinucleotide with ammonium hydroxide in methanol at ambient temperature over night. This way, the heteroporphyrin dinucleotide $dU^{ZnDPP}dU^{ZnTPP}$ **2** and the homoporphyrin dimer $d(U^{ZnTPP})_2$ **3** were obtained in moderate yields of about 5 to 10%.

In order to test the ability of the phosphoramidite under solid-phase synthesis conditions, the 5'-DMT protected phosphoramidite nucleotide **4** (Scheme 2) was synthesised and obtained in about 60% yield after column chromatography. By using the universal solid support II (15 μmol) provided by Glen Research, the synthesis was performed following standard procedures for deprotection, oxidation, and capping in an automated DNA synthesizer. However, the coupling of the building blocks to the solid support and the formation of the internucle-

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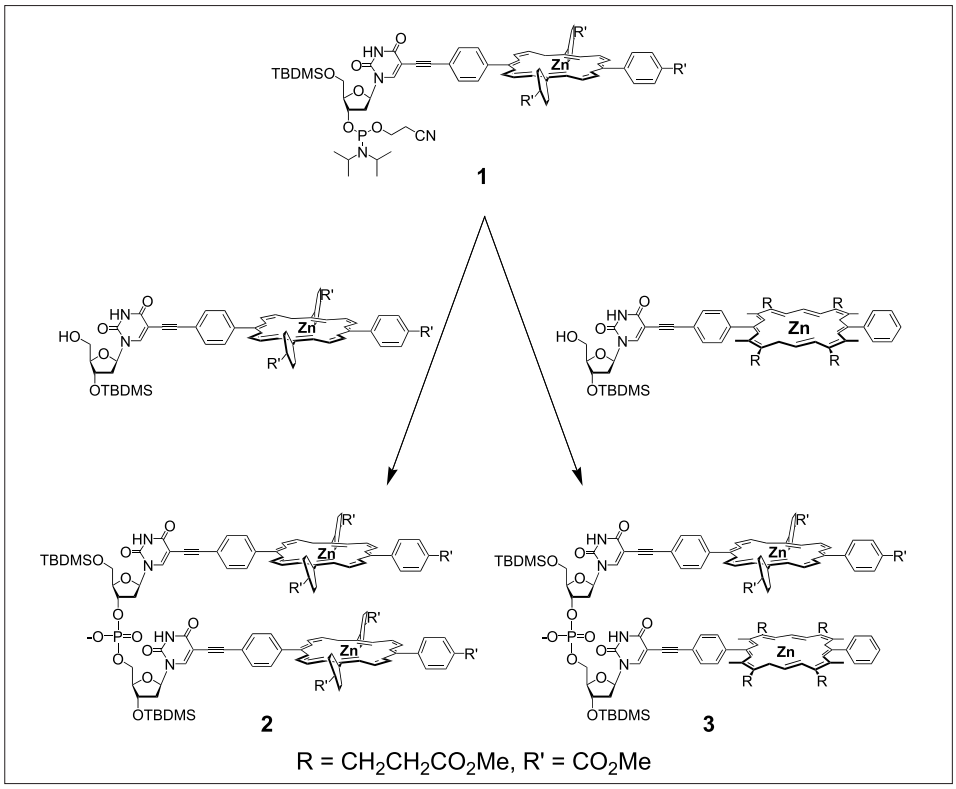
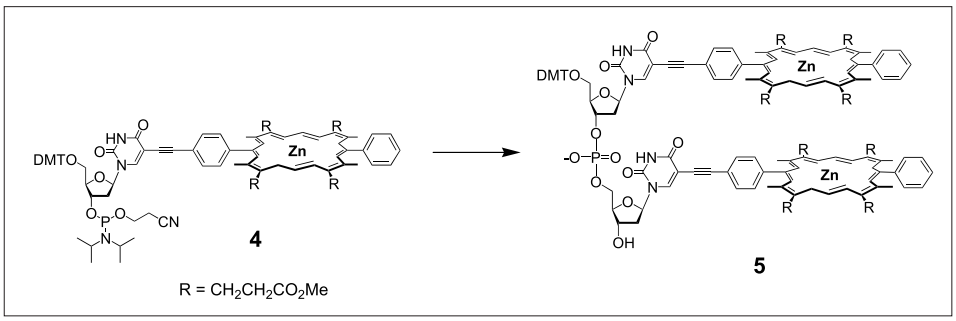


Scheme 1.



Scheme 2.

osidic linkage were done manually outside the synthesizer. For this purpose, the solid phase was initially rinsed with the standard activator solution (tetrazole in acetonitrile), and then a mixture of 1 ml activator and 0.5 ml phosphoramidite solution (0.15 mM in DCM) were passed back and forth through the column for 20 min with the aid of two syringes. The DMT protecting group was left on the 5'-end of the dinucleotide for solubility reasons. Cleavage from the solid support and deprotection of the phosphate group was performed with ammonium hydroxide (25% aq. soln./methanol 3:1) at ambient temperature over night. After washing of the resin with methanol and DCM, the homoporphyrinic dinucleotide was purified on a sephadex LH20 column. Initially, the dimer is obtained as mixed free base and zinc porphyrin due to decomplexation of the first coupled porphyrin during DMT deprotection. The dimer was accordingly re-metallated with zinc acetate in $CHCl_3$/methanol, and after a second column on sephadex obtained in pure form as $d(U^{ZnDPP})_2$ **5**. According to trityl monitoring, the coupling efficiency was in the order of 90%; however, the initial loading of the solid support seemed far less than anticipated and might explain the low yield of less than 1 mg.

The UV-Vis absorption spectra of the dinucleotides are shown in Fig. 1 (solid lines), together with the calculated spectra obtained from a superposition of the individual building blocks (dotted lines). The data are compiled in the Table. It should be noted that an accurate measurement of the extinction coefficients of **5** was not possible due to the low amount of material obtained; however, from the absorbance we estimate the solution to be around 10^{-6} M in **5**. A comparison of the calculated and measured spectra reveals that the absorption maxima of the dinucleotides do not exactly match with the calculated spectra, thus electronic interactions between the chromophores occur. The strength of the interaction is highly dependent on the structure of the porphyrins in the dimers. Slight blue shifts are observed in the case of **2** ($\Delta\lambda$ ~1 to 2 nm). The steric hindrance due to the *meso*-phenyl groups obviously does not allow the porphyrin cores to approach close enough to induce strong electronic coupling. The mixed dimer **3** shows a spectrum which differs greatly from the calculated spectrum of $dU^{ZnTPP}dU^{ZnDPP}$: the absorbance seems to be dominated by the electronic properties of the TPP part, and small red shifts for the B-band and Q-band absorptions are observed. In the DPP homodinucleotide **5**, the B-band absorption at 419 nm is marginally shifted by about 3 nm to lower energy, but in the Q-band region an additional peak at 629 nm is observed. This additional absorption maximum is not due to incomplete metallation, because in the mixed free base–zinc dinucleotide originally isolated three additional weak maxima at 648, 711, and 740 nm are visible (data not shown), which are absent in **5**. Also, the free base DPP does not exhibit any absorbance in this region [3].

Upon titration of a saturated methanolic solution of the complementary diadenosine into the solutions containing the porphyrin dinucleotides, the absorbances gradually changed. The final spectra are also displayed in Fig. 1 (dashed lines). After this point (*ca.* 60 µl $d(A)_2$ solution in 1.5 ml dinucleotide soln.), the absorbances decreased due to dilution. In the case of **2**, the extinction coefficients increased slightly, but again the shifts of the absorption maxima hardly changed. For the duplex $d(A)_2$-**3**, however, large blue shifts in both the B-band and the Q-band absorption maxima are observed (Table), and the values for λ_{max} are in-between those of **2** and **5**. The titration experiment with **5** resulted in no change of the spectrum. Since **5** was not soluble in pure $CHCl_3$ unlike **2** and **3**, a minimum of 10% of methanol was necessary to solubilise **5**. Thus, the duplex formation with $d(A)_2$ was suppressed due to the high content of the protic solvent which normally destabilises hydrogen-bonded systems.

From a simple model point of view, it seems that in the dinucleotides the two chromophores can approach sufficiently close to show electronic interactions, which are stronger in the case of the sterically less hindered DPP derivatives **3** and **5**. Upon formation of the helical duplex $d(A)_2$-**3**, the porphyrins might be rotated away from each other, and the weaker electronic coupling results in an overall blue shift of the absorbance of the dimer. However, to determine the exact nature of the interactions more spectroscopic investigations will be necessary. In the case of $d(A)_2$-**2**, no significant changes are observed. Thus, in the case

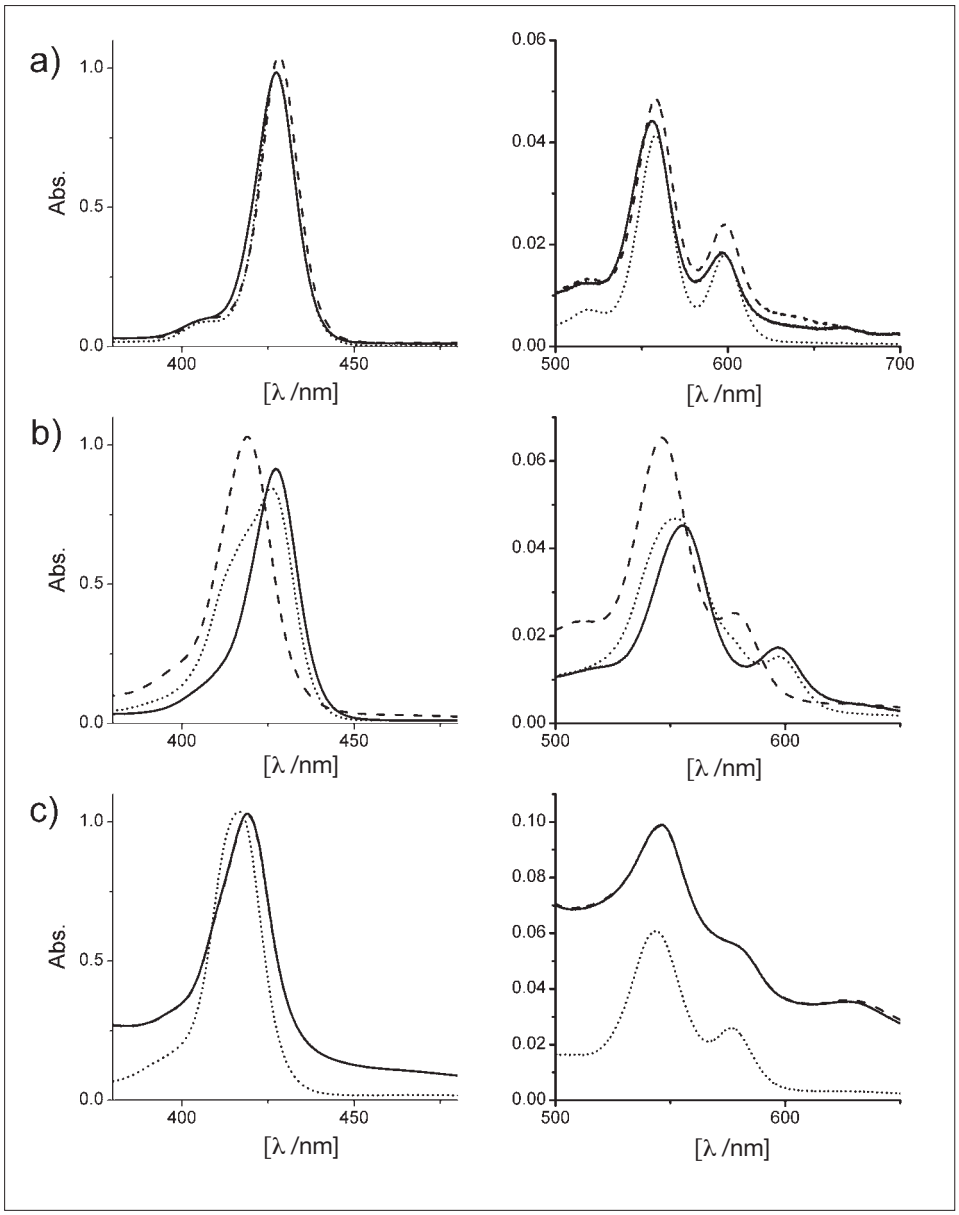


Fig. 1. UV-Vis absorption spectra of the dinucleotides (solid lines), the duplexes with the complementary $d(A)_2$ (dashed lines), and of the calculated spectra of the dimers (dotted lines). The measurements were performed in $CHCl_3$ solutions (10^{-6} M) except c) (cf. text). a) **2**; b) **3**; c) **5**.

Table. Absorption maxima λ_{max} (log ε) of the dinucleotides, the duplexes with $d(A)_2$, and of the calculated spectra obtained by addition of the building blocks. Measurements were performed in $CHCl_3$ (10^{-6} M, 25 °C) except **5** (cf. text).

	B-band	Q-bands		
2	427 (5.96)	556 (4.65)	598 (4.24)	
$d(A)_2$-**2**	428 (6.01)	558 (4.81)	598 (4.40)	
$d(^{UZnTPP})_2$ calcd.	428 (5.93)	559 (4.67)	598 (4.18)	
3	427 (5.96)	555 (4.65)	598 (4.24)	
$d(A)_2$-**3**	418 (6.01)	546 (4.81)	578 (4.40)	
$dU^{ZnTPP}dU^{ZnDPP}$ calcd.	426 (5.93)	551 (4.67)	597 (4.18)	
5	419	546	577 (sh)	629
$d(U^{ZnDPP})_2$ calcd.	416	543	577	

of the TPP homodinucleotide the electronic features of the porphyrins are additive.

In the MALDI-TOF mass spectrometer, irradiation with the laser induced photolytic fragmentation of the dinucleotides, probably due to radicals generated from the photoexcited state of the porphyrins. We had previously observed cleavage of functional groups and atom transfer in MALDI-TOF MS [6]. Suppression of this fragmentation could be achieved to some extent by varying the matrix used [7], and most usefully proved to be a combination of dihydroxy acetophenone together with *p*-nitro aniline. When the dinucleotides are mixed with an excess (50 equiv.) of the complementary $d(A)_2$, formation of the duplex is also observed. The duplex formation is best seen in the case of **3**, where the complex $d(A)_2$-**3** was clearly detectable in the mass spectrum (Fig. 2). For **2** and **5**, the complexation was much less obvious, and especially $d(A)_2$-**5** displayed m/z peaks corresponding to multiple salt adducts.

In summary, we have presented the synthesis of porphyrin dimers based on dinucleotide formation, where the building blocks are connected through a phosphate diester backbone in analogy to the natural DNA structure. The general route to substituted nucleosides *via* Sonogashira coupling allows introduction of a much larger diversity of structurally different porphyrins, and the mode of connection is independent of the substitution pattern of the porphyrin. Both solution- and solid-phase synthesis can be applied, and simply by varying the building blocks the composition of the dimer can be freely chosen. Despite the overall low yield, solid support synthesis is advantageous due to the much easier purification of the product. As the first spectroscopic investigations demonstrate, it should be possible to modulate the electronic properties of larger multiporphyrin arrays obtained by this strategy. Both UV-Vis spectroscopy and mass spectrometry reveal that the presence of two consecutive porphyrins does not hinder the specific recognition of the complementary nucleobase, thus multiple incorporation of the building blocks into DNA should not prevent the formation of the double-stranded helix of the modified DNA with its complementary strand. We are currently evaluating the physical properties of such a porphyrinyl-DNA.

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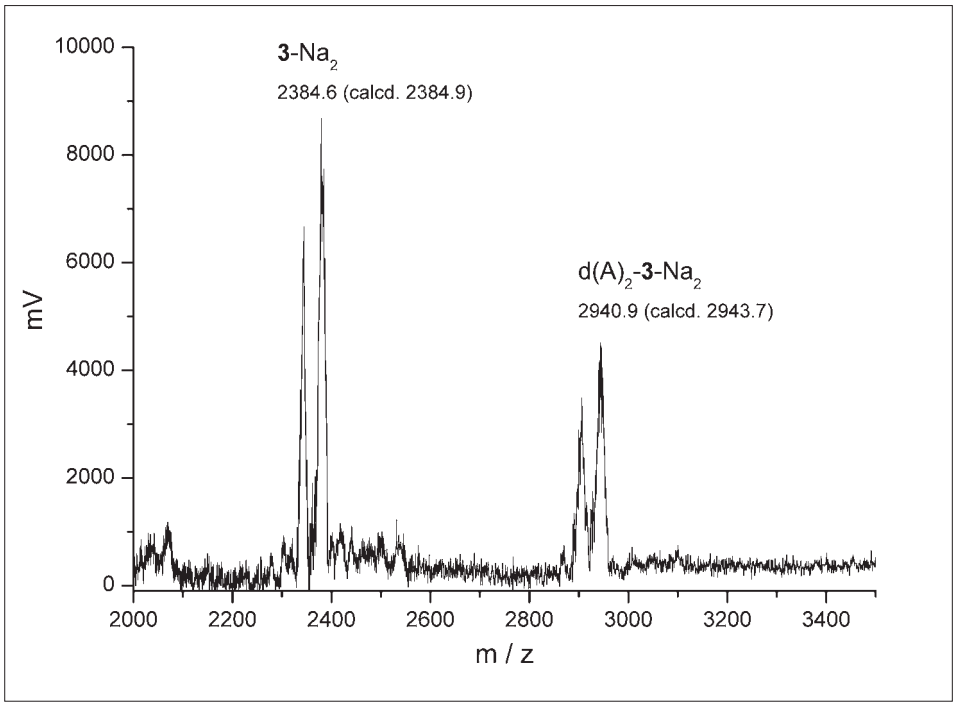

Fig. 2. MALDI-TOF MS of a mixture of **3** and $d(A)_2$.

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