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A Porphyrin *C*-Nucleoside Incorporated into DNA

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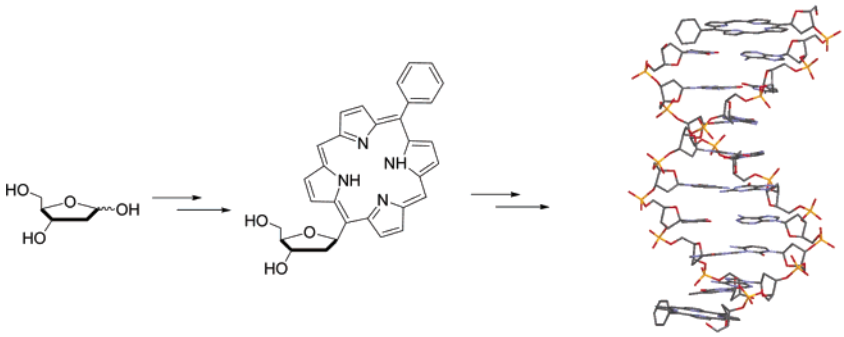
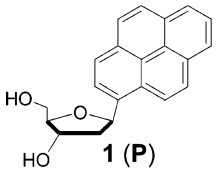
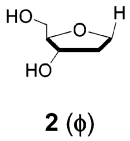
ABSTRACT



A free porphyrin coupled on 2-deoxy-D-ribose was synthesized and incorporated into DNA via phosphoramidite chemistry. Substitution at the ends of a 5′-modified self-complementary duplex was found to be thermally and thermodynamically stabilizing. The porphyrin moiety strongly intercalates in the duplex when located near the center, and retains its fluorescence properties in DNA.

The strategy of replacing DNA natural bases by nonpolar surrogates[1,2] has opened ways to add new functions to the DNA structure, to probe protein−DNA interactions,[3] to stabilize structure,[4] and to probe the internal features of the helix.[5] Simple aromatic hydrocarbons often add stability to a duplex when located at the end positions.[6] At the interior of the helix, nonpolar nucleosides are in some cases destabilizing;[1a,d] however, if their stacking and/or size are sufficient, robust non-hydrogen-bonded base pairs with stabilities challenging those of natural pairs can be formed.[7] Pyrene nucleoside (**1**) paired opposite an abasic nucleoside (**2**) is a remarkable example of a non-hydrogen-bonded pair that is both stable in DNA[7a] and also efficiently and selectively processed by polymerase enzymes.[8]





Many of these aromatic hydrocarbons are also fluorophores,[9] and nucleosides such as **1** (and its α-epimer) have

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found use as fluorescent reporters in biophysical[3c,d,5a] and biosensing experiments.[10] With an ongoing interest in finding new DNA base replacements with varied fluorescence characteristics we turned our attention to porphyrins, which are flat aromatic heterocycles with unique and highly tunable chemical and luminescent properties.[11]

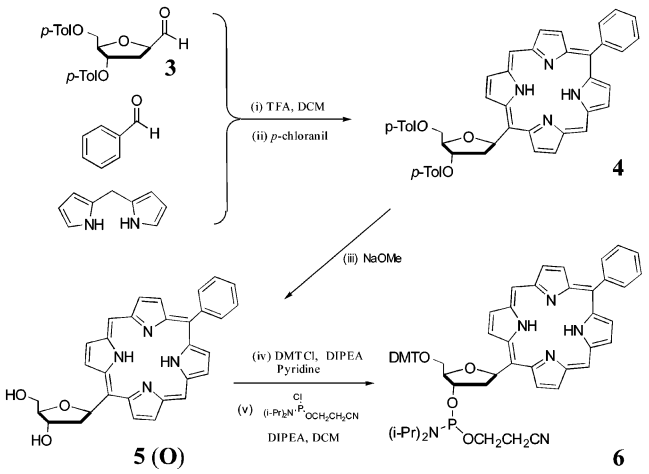
Extensive previous work on porphyrin−DNA interactions has focused on synthetic cationic water-soluble porphyrins, which depending on the structure and the presence of coordinated metals are able to interact with DNA in three different modes: intercalation, outside groove binding, and outside binding with self-stacking.[12] This binding, however, is driven primarily by electrostatic attractions between the negatively charged DNA backbone and the cationic porphyrin residues rather than direct interaction of the porphyrin ring with the nucleobases. Various cationic porphyrin moieties have been conjugated to oligonucleotides to increase the binding selectivity; recent examples have included neutral porphyrins with an entirely hydrophobic core conjugated to the 3′ terminus of oligonucleotides using flexible linkers[13] or internally using non-ribose tethers.[14] Although the hydrophobicity of the porphyrin ring might support close interaction with neighboring bases, the use of linkers or tethers creates uncertainty as to the location of the porphyrin within the conjugated oligonucleotide or in its interaction with complementary DNA strands.

We set out to synthesize a porphyrin macrocycle directly attached to the natural backbone of DNA, via a C−C bond at the anomeric position of deoxyribose. Such a design allows for a more rigid and intimate location of the porphyrin with the DNA. In addition, use of this derivative enables incorporation into oligonucleotides at *any position* using standard phosphoramidite chemistry and automated DNA synthesis. Herein, we report the synthesis and properties of this novel *C*-porphyrinyl nucleoside, its facile incorporation into oligonucleotides, its fluorescence, and preliminary thermodynamic data for duplexes containing this moiety.

While incorporation of aromatic groups to form aryl *C*-nucleosides into the anomeric position of the 2-deoxy-D-ribose unit has been achieved via nucleophilic attack of aryl organometallics to Hoffer's α-chlorosugar[1c,9] (3,5-di-*O*-toluoyl-α-1-chloro-2-deoxy-D-ribofuranose) and ribolactone precursors,[15] a free-porphyrin does not undergo selective formation of nucleophilic Grignard or organolithium reagents.[16] Therefore, our approach involved assembly of the porphyrin *de novo* on the sugar moiety.[17]

A 3,5-bis-*O*-toluoyl-protected deoxyribose-C1-carboxaldehyde (**3**) was prepared in three steps from Hoffer's α-chlorosugar via a nitrile glycoside isolated in the *β* configuration.[18] A mixed aldehyde condensation of **3** with benzaldehyde and dipyrromethane under Lindsey conditions for meso-substituted porphyrins[19] afforded a mixture of porphyrins from which a trans-substituted 5,15-phenylporphyrin nucleoside **4** was isolated in 15−20% yields.[20]

Scheme 1



Deprotection of the sugar moiety gave *C*-porphyrinyl nucleoside **5** as a glassy purple solid. Structural characterization was performed by means of ^{1}H, ^{1}H-COSY, and NOE NMR spectroscopy. The chemical shifts for the ribose unit in **5** were displaced downfield in comparison with the observed values in other *C*-nucleosides.[6,9] In particular, the 1′ proton typically found between 5 and 6.5 ppm with polycyclic aromatic hydrocarbons[9] exhibited a strong deshielding to 8.3 ppm in pyridine-d_5. Confirmation of the *β*-glycosidic configuration of **5** was obtained by NOE difference experiments. Irradiation of the 1′H gave NOE on 4′H (6.2%) and 2′H-α (7.3%). Similarly, irradiation on 2′H-*β* gave NOE on 3′H (5%) and the neighboring pyrrolic-*β*-H (7.8%) whereas irradiation at the 2′H-α only yielded significant enhancements on 1′H (6.3%).

β-*C*-Porphyrinyl nucleoside (abbreviated **O**) displays the standard features of 5,15-disubstituted porphyrins, with UV−

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(16) Radical anions are formed by abstraction of a proton at meso or *β* positions.

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(20) In principle three porphyrins can be formed. 5,15-Diphenylporphyrin was isolated in 10−15% yields. A third compound, presumably the porphyrin bearing two ribose units, was observed on TLC but not isolated.

vis absorption characterized by a strong band at 400 nm (Soret, $\epsilon_{400} = 3.24 \times 10^5$ M^{-1} cm^{-1}) and four weak absorptions in the region between 500 and 650 nm (Q-bands, $\epsilon_{500} = 1.6 \times 10^4$ M^{-1} cm^{-1}). Likewise, the steady-state fluorescence shows large Stokes shifts with emission bands at 629 and 694 nm (λ_{exc} 400 nm) and quantum efficiency of 0.11 in MeOH.

Nucleoside **O** was further 5′-*O*-tritylated and 3′-*O*-phosphitylated to afford 2-cyanoethyl phosphoramidite **6** in 50% overall yields. With compound **6** in hand, synthesis of oligonucleotides containing **O** was readily performed by standard automated DNA synthesis methods (see Table 1).

Table 1. Fluorescence Emission and Quantum Yield Data for Oligonucleotides Containing **O** in Water at 25 °C

		λ_{abs}[a]	λ_{em}[b]	Φ[b]
5	**O** (MeOH)	400	629	0.110
7	5′-$(\phi)_9$**O**	400	622	0.082
8	5′-**O**ACAGCTGT	405	630	0.106
9	5′-CTTTTC**O**TTCTT	408	632	0.108
10	5′-AAGAA**O**GAAAAG	408	631	0.102

[a] Soret band, nm. [b] Absorbance of solutions <0.05, λ_{exc} 405 nm. Quantum yield estimated by ratio with tetraphenylporphine in duplicate essays. Error ±10%.

Derivative **7**, a water-soluble oligomer containing abasic nucleosides terminated with the porphyrin nucleoside (ϕ_9**O**), displayed identical absorption spectra as the parent **O** in MeOH, and a small blue shift in the fluorescence emission. In the presence of nucleobases, the Soret band was red-shifted by 5 nm in **8** (facing a single base) and 8 nm in **9** and **10**, interacting with two bases. Surprisingly, apart from the small red shift displayed in the emission band of **8**, **9**, and **10**, in comparison to **7**, the quantum efficiency of **O** seems to be independent of the presence of nucleobases. DNA interaction with fluorescent dyes often produces quenching of fluorescence.[21] For instance, in cationic porphyrins substantial quenching was observed in intercalative binding at GC regions as a consequence of deactivation of an excited-state complex with G via reductive quenching of the porphyrin excited state.[22]

We evaluated the stability of self-complementary duplexes d(**X**ACAGCTGT)$_2$ containing **X** as natural bases and **O** by thermal denaturation (Table S3). This sequence containing natural bases as dangling ends was previously shown to display a two-state duplex to coil transition without formation of hairpin or slipped-duplex conformations.[23]

Duplexes with **O** showed a significant increase in the melting temperature ($\Delta T_m = 14.7$ °C) relative to the core sequence, about twice the effect of T or A (7.2 and 8.1 °C, respectively) (Table S3). In contrast to this strong thermal stabilization provided by **O**, its thermodynamic stabilization ($\Delta\Delta G_{37} = -0.60$ kcal/mol per residue) is similar to the stabilization with dangling A base. The slope of the melting curve with dangling **O** was less steep than duplexes with natural bases, indicative of lower cooperativity (Figure S4). Although **O** is larger than the pyrene glycoside **P**, it provides somewhat less thermal and thermodynamic stabilization. Models suggest that **O** may have a geometry that provides less surface area of overlap with neighboring bases (Figure S5).

Phosphoramidite **6** allowed ready access to oligonucleotides in which **O** could be located internally in the duplex. Models of duplex DNA (B-form) indicated that a porphine macrocycle (surface area, 327 Å^2), despite its larger surface area in comparison to thymine−adenine pair (surface area, 269 Å^2), can be accommodated within a DNA duplex.[24] In **O**, however, a phenyl group is located opposite to the ribose in the porphyrin. From the synthetic perspective, introduction of the phenyl group facilitated both synthesis and isolation of these compounds. However, as DNA base surrogate, this additional feature might be expected to compromise duplex formation and stability. However, models showed the phenyl group in **O** pointing outward in the major grove of the duplex with no interference with neighboring base pairs or the sugar−phosphate chain.[24]

We tested the stability and pairing ability of **O** in 12mer duplexes (Table S4) by thermal denaturation. Duplexes containing the four natural bases and the abasic dideoxyribose **2** (abbreviated ϕ) paired opposite **O** in sequence **9** showed destabilization of 8 to 10 °C in T_m and 2.5−3 kcal/mol in ΔG_{25} relative to the natural T−A base pair control duplex. Similar results were obtained with **9** paired against a mismatched sequence (A deleted). Although destabilizing overall, the data suggest that internal substitution of **O** must help to maintain a continuous stacking in both strands given that significant stabilization ($\Delta T_m \sim 10$ °C and $\Delta\Delta G_{37} \sim 2.5$ kcal/mol) was gained in comparison to duplexes containing natural bases opposite an abasic site (Table S4).

CD studies of duplexes containing **O** in the same buffer revealed spectral features of B DNA similar to the control T−A duplex (Figure 1). Moreover, a strong negative induced CD signal in the Soret absorbing region was consistent with full intercalation of the porphyrin.[25] By contrast, single stranded **9** displayed a strong positive induced CD band.

The temperature dependence of the CD spectrum of a duplex containing **O** paired with the abasic site (Figure 2) clearly showed the transition from duplex to single strand concomitant with the loss of the negative CD band and appearance of a positive band upon increasing the temperature. The results overall seem to indicate a strong preference of **O** to bury its hydrophobic surface within a more favorable environment even at the expense of duplex formation and stability. This behavior may also account for the poor stability

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(25) Large negative induced CD signals have been associated with intercalative binding to DNA by cationic porphyrins. See ref 12.

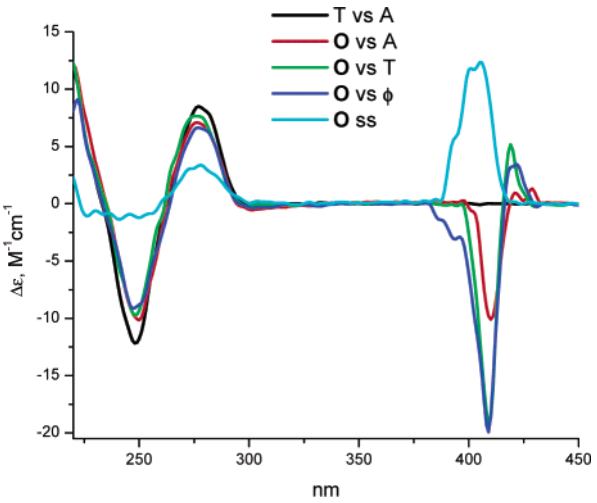

Figure 1. Circular dichroism of duplexes containing **O** paired against A, T, and ϕ. Also shown are control duplex (T opposite A) and single stranded **9**. Conditions are similar to those in Table S4 at 20 °C.

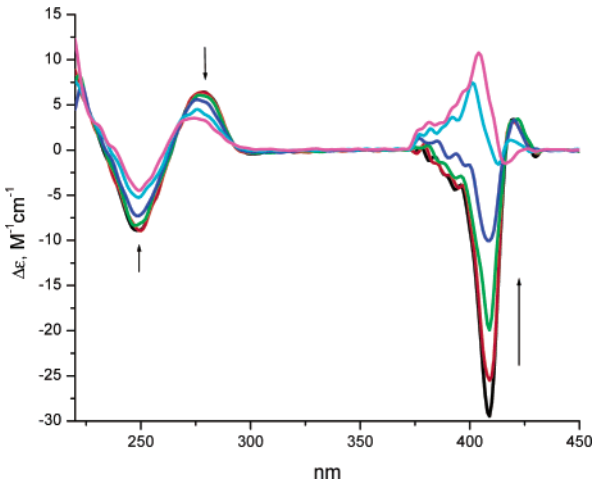

Figure 2. Temperature dependence of circular dichroism spectra of a duplex containing **O** paired against ϕ. Conditions were similar to those in Table S4. Arrows indicate changes as temperature was raised from 5 to 50 °C.

observed in duplexes with **10**, containing **O** in the purine-rich strand.

In summary, we have demonstrated a convenient route to incorporate a porphyrin macrocycle at any position in oligonucleotides. This allows a more precise location of the porphyrin moiety in duplex DNA. In addition, designed structures with **O** located as dangling terminus can provide substantial stabilization of the helix. Other effects on stacking such as sequence dependence and the influence of metal cations also could be of interest.

Unlike many fluorophores such as pyrene, the fluorescence properties of the porphyrin are not strongly affected by the presence of nucleobases both in single stranded and in duplex structures (see Supporting Information). This enhances its utility in labeling of DNA. Beyond that application, we foresee several other uses for this nucleoside given the widespread interest in porphyrins in the design of biomimetic structures,[26] supramolecular devices and materials,[27] sensors,[28] light-harvesting arrays,[26] and probes for electron transfer in DNA.[29]

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Supporting Information Available: Experimental procedures and characterization of **4**, **5**, and **6**; figures containing absorption and fluorescence spectra of **7**−**10**; Tables S1 to S4 including thermodynamic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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