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Porphyrin conjugated to DNA by a 2′-amido-2′-deoxyuridine linkage

Sarita Sitaula and Scott M. Reed*

Department of Chemistry, Portland State University, PO Box 751, Portland, OR 97207, USA

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Abstract—A porphyrin that contains a single carboxylic acid group was synthesized and coupled to 2′-amino-2′-deoxyuridine. The resultant product contained a free 3′ hydroxyl group and a 4,4′-dimethoxytrityl (DMT) protecting group on the 5′ hydroxyl of the uridine, making it suitable for use in oligonucleotide synthesis. The 3′ H-phosphonate derivative of this molecule was synthesized and used to form a conjugate with a 19 nucleotide sequence of DNA (5′-CCTCCAGTGGAAATCAAGG-3′). This was carried out with the DNA attached at the 3′ end to a control pore glass (CPG) substrate, allowing for rapid purification. After removal of the DMT group, an additional three nucleotides were added, leaving the porphyrin as an internal modification. This is the first report of porphyrin attached internally to an oligonucleotide using a hydrogen-bonding nucleoside analog. This allows oligonucleotides to be used as a scaffold for precise positioning of multiple porphyrins within biomimetic arrays.

Porphyrins perform a wide variety of functions in natural and synthetic systems. They have been used in light-activated cancer treatment,[1] are the functional element of light harvesting systems,[2] and have been used in sensor design due to their fluorescent and electrochemical properties.[3,4] Arrays that contain multiple porphyrins are also of interest in biological and synthetic systems since unique catalytic and photonic properties arise when porphyrins are held in close proximity to each other.[5–10] For example, the light-harvesting complex of photosystem II contains a number of porphyrinic chlorophyll and xanthophyll molecules spatially arranged to optimize light collection and energy transfer.[11,12]

Synthetic chemists strive to mimic the function of these complex biological systems and oligonucleotides can provide an organized scaffold for precise positioning of porphyrins within arrays. Two general strategies have been employed for conjugating porphyrins to oligonucleotides. In the first approach a porphyrin is coupled after the oligonucleotide has been synthesized and removed from a solid-phase support.[13] Typically this is accomplished by synthetic incorporation of a nucleophilic site at either the 3′ or 5′ end of the oligonucleotide that is subsequently coupled to an activated porphyrin. A second approach is to perform the porphyrin conjugation while the oligonucleotide remains on the solid-phase support. This approach simplifies purification and is advantageous in driving reactions to high yield. In one example, a 5′ porphyrin–oligonucleotide conjugate was synthesized on a control pore glass (CPG) support by an H-phosphonate method.[14] In another example, porphyrins have been conjugated to the 3′ and 5′ ends of oligonucleotides using phosphoramidite chemistry.[15,16] In each of these approaches, only a single porphyrin can be added to the oligonucleotide chain. To fully employ oligonucleotides as a scaffold for constructing arrays of multiple porphyrins, modification at internal positions without interrupting base pairing is necessary. Methods of internal modification have been reported, however, the modifiers that were used lacked hydrogen-bonding nucleosides and therefore are incapable of base pairing.[17]

A promising new route to internal modification of oligonucleotides is accomplished using a modified nucleoside that contains a 2′ amine group, a free 3′ hydroxyl group, and a 4,4′-dimethoxytrityl (DMT) protected 5′ hydroxyl group.[18] 2′-Amino-2′-deoxyuridine provides a convenient route for modification of the deoxyribose ring through coupling to a nucleophilic amine.[19] This approach avoids disruption of the hydrogen-bonding base

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* Corresponding author. Tel.: +1 503 725 8512; fax: +1 503 725 9525; e-mail: sreed@pdx.edu

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by attachment to the 2′ position on the deoxyribose ring. In order to use aminouridine derivatives in an automated DNA synthesizer the 3′ hydroxyl is converted to an H-phosphonate or phosphoramidite derivative. 3′ Phosphoramidites of 2′ amido-modified uridine,[20,21] adenosine,[22] and guanine[23] have been previously reported. H-phosphonate chemistry has been used to synthesize DNA[24] and to modify oligonucleotides with porphyrins,[14] flavins,[25] and a benzo[*a*]pyrene derivative.[26]

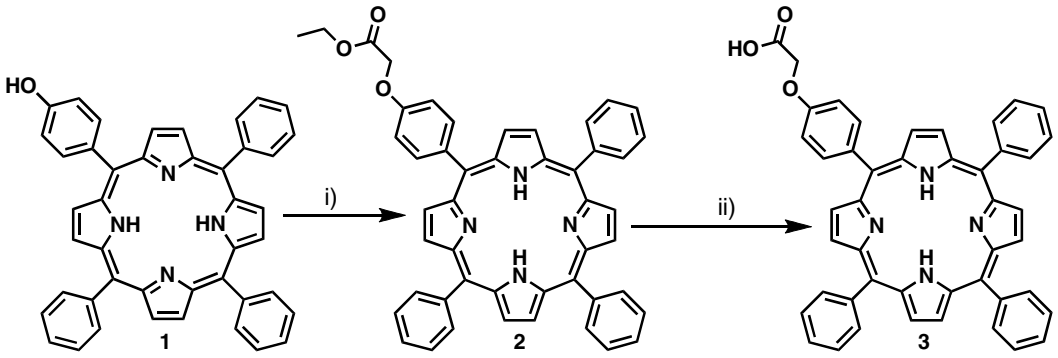
A novel route is described here to modifying DNA using a porphyrin coupled to the nucleoside, 2′-amino-2′-deoxyuridine. The synthesis of a 2′-amide linked porphyrin–uridine conjugate is reported and a method for activating it at the 3′ position using H-phosphonate chemistry is described.[27] Attachment of the porphyrin at the 2′ position leaves the 5′-DMT available for further reactions. Nucleotides modified at the 2′ position with aromatic groups tethered by appropriate length linkers have been shown to increase rather than disrupt base-pairing strength in DNA duplexes.[28] Furthermore, we demonstrate that this 3′ H-phosphonate can be used in solid-phase DNA synthesis by coupling it to a 19-mer DNA. Use of a 5′-DMT allowed for oligonucleotide extension after deprotection, leaving the porphyrin as an internal modification after continued synthesis. This approach allows for the inclusion of additional bases or additional modifiers (e.g., thiol, amine, or biotin) beyond the nucleotide that contains the porphyrin. Another benefit of this route is that it can accommodate porphyrins with different core structures,[1c] functional groups on the phenyl rings of the porphyrin, and linkers of various lengths to the oligonucleotide.[15,28]

Synthesis[29] of the 2′ porphyrin modified uridine started with preparation of porphyrin **1**, functionalized with a single hydroxyl group. Tetraphenyl porphyrin and its derivatives are commonly used as a starting point for the design of biomimetic porphyrin arrays[5] due to their synthetic tunability. By varying the functional groups on the peripheral phenyl rings of tetraphenyl porphyrin it is possible to alter the solubility and reactivity of the porphyrin without disrupting its interesting electronic and optical properties. A porphyrin functionalized with a single hydroxyl group, 5-(4-hydroxyphenyl)-10,15,20-triphenyl porphyrin, was synthesized by modification of literature procedures.[30,31] Compound **1** can be synthesized as described in Ref. 32 or by adapting a low-solvent, microwave method.[30] Compounds **2** and **3** were synthesized as shown in Scheme 1 by modification of a literature procedure for the conversion of tetra(hydroxyphenyl) porphyrin to tetra(carboxyphenyl) porphyrin.[32]

Compound **1** (30 mg, 0.047 mmol) was dried in vacuo in a 100-ml flask and flushed with nitrogen. Potassium carbonate (315 mg, 2.27 mmol) and potassium iodide (44 mg, 0.261 mmol) were added to the flask under nitrogen. Nitrogen-sparged DMF (0.5 ml) and CH_2Cl_2 (4 ml) were added. Ethylchloroacetate (100 μl, 0.704 mmol) was injected into the flask under nitrogen, the flask was fitted with a condenser and was refluxed for 5 h. Solvent was removed in vacuo. After removal of DMF the reaction mixture was extracted with CH_2Cl_2, washed with water (3× 20 ml), dried over magnesium sulfate, and the solvent was evaporated to yield ethyl-5-(4-carboxylatomethoxy),10,15,20-triphenylporphyrin **2** as a purple solid (32.3 mg, 95.8%). ^{1}H NMR and UV/vis spectra matched literature values.[31]

Conversion of the ethyl ester **2** to the carboxylic acid **3** was performed under basic conditions as shown in Scheme 1. Compound **2** (50 mg, 0.069 mmol) was added to a flask followed by 10% potassium hydroxide in 1:1 ethanol/water (30 ml) and refluxed for 18 h. The mixture was cooled to room temperature and diluted with water (10 ml). Afterward, HCl (0.1 M) was added dropwise until the solution became neutral. CH_2Cl_2 (50 ml) was added and the organic layer was separated, washed with water, and dried with magnesium sulfate. After removal of the solvent in vacuo, 5-(4-carboxylatomethoxy)-10,15,20-triphenyl-phenylporphyrin **3** was obtained as a purple solid (33 mg, 69.5%).[33]

The resultant carboxylic acid functionalized porphyrin was coupled to 2′-amino-2′-deoxyuridine to prepare **4** as shown in Scheme 2. 2′-Amino-2′-deoxyuridine was synthesized following a literature procedure.[20] EDCI (5.0 mg, 0.026 mmol), **3** (13 mg, 0.018 mmol), 2′-amino-2′-deoxyuridine (9 mg, 0.017 mmol), and DMAP (<1 mg) were added to a 25-ml flask. The flask was fitted



Scheme 1. Synthesis of porphyrin **3**. Reagents and condition: (i) $ClCH_2COOC_2H_5/KI/K_2CO_3$ in DMF/CH_2Cl_2; (ii) 10% KOH in ethanol/water (1:1)/reflux.