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# Synthesis and characterization of flavin-tethered peptide nucleic acid

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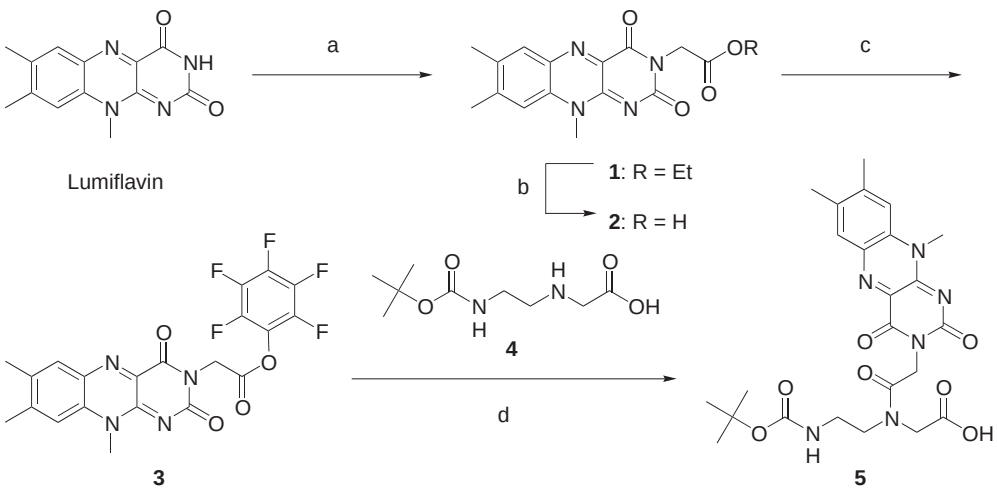
**Abstract**—We synthesized flavin-containing PNA monomer unit **5** from lumiflavin and prepared PNAs containing a flavin moiety (FPNA) by the standard *t*Boc chemistry. Each PNA oligomer was purified by reversed-phase HPLC and characterized by MALDI-TOF MS and UV spectra. Thermodynamic analyses indicated that the PNA oligomer containing a flavin moiety near the amino terminal considerably stabilized the PNA–DNA hybrids. 

PNA is an artificial biopolymer in which the DNA sugar-phosphate backbone is replaced by a peptide backbone.[1] It has several advantages over natural nucleic acids: (i) PNA oligomers can be easily synthesized by solid-phase *t*Boc or Fmoc chemistry,[2] (ii) are extremely stable to cellular nucleases and proteases,[3] and (iii) can hybridize with complementary DNA with high affinity.[4] However, it has been known that there is a serious limitation to the uptake of PNA into cells.[5] Modification of PNA backbones has been examined directed toward the improvement of their binding to DNA and/or RNA and of the membrane permeability.[6] Simultaneously, several functionalized PNA oligomers, which contain functional molecules in place of native nucleobases, have been designed for the improvement of water solubility and for the study on electron transfer chemistry through a DNA duplex.[7]

DNA photolyase is well-known to photoconvert the cyclobutane ring of a pyrimidine dimer directly to the




**Scheme 1.** (a) Ethyl bromoacetate, potassium carbonate, DMF, rt, 2 days, 85%; (b) conc. HCl, reflux, 6 h, 99%; (c) pentafluorophenol, EDCI, DMF, 0°C, 1 h then rt, 2 h, 85%; (d) **4**, diisopropylethylamine, DMF, rt, 15 h, 96%.

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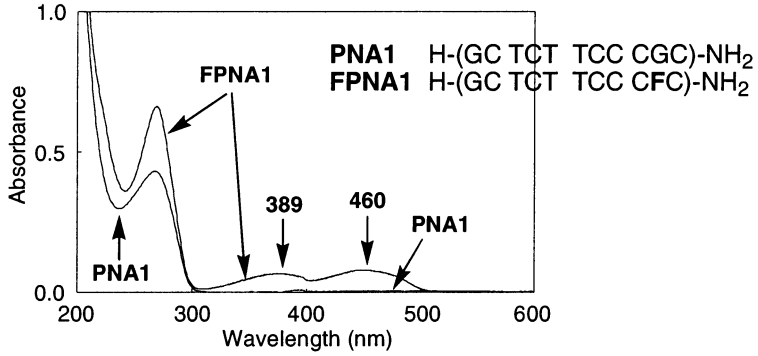


**Figure 1.** UV spectra of **PNA1** and **FPNA1**. Concentration of single stranded PNA was 5 μM (strand concentration) in 50 μM sodium cacodylate (pH 7.0). Bold **F** means flavin PNA unit.

two corresponding pyrimidines without the nucleotide excision.[8] Recently, flavins, which are chromophores of DNA photolyases,[8,9] have been investigated for the mechanism of the DNA repair system.[10] Also, an attempt to synthesize a flavin-containing DNA oligomer as an antisense molecule has been examined.[11] However, flavins catalyze the conversion of phosphoramidite to phosphoramidate via intramolecular photosensitized oxidation during the amidite synthesis, implying that the synthesis of flavin-containing DNA oligomers can be hardly synthesized by conventional methods. We now wish to report herein the synthesis and characterization of a flavin-tethered PNA.

Flavin-containing PNA monomer unit **5** was prepared from lumiflavin (Scheme 1).[12] The facile alkylation of the *N*-amino group of lumiflavin with ethyl bromoacatate (**1**) followed by acid hydrolysis of the resulting ethyl ester gave **2**. Flavin is known to be easily decomposed by alkaline hydrolysis.[13] PNA monomer **5** was prepared from the active ester **3** and *N*-(2-Boc-aminoethyl)glycine **4**.[14] It is possible to know whether a flavin moiety is incorporated into PNA oligomers by simply measuring their UV spectra because UV absorption of **5** at 390 and 460 nm is not overlapped with those of any PNA nucleobases, as shown in Fig. 1.[15]

Several PNA oligomers were synthesized by solid-phase *t*Boc chemistry as previously described.[16] A Ninhydrin test showed that each coupling of PNA monomers with MBHA resin using HBTU proceeded quantitatively. After completion of PNA oligomer elongation, the resin was treated with TFA/TFMSA/thioanisole/*p*-cresol (6:2:1:1) for release of the PNA oligomer from the resin and for deprotection of the carbobenzyloxy and Boc groups. After the acid solution was filtered off to remove the MBHA resin, the supernatant was poured into ether, and then the precipitate was collected by centrifugation. The residue was redissolved in TFA, and the solution was reprecipitated in ether and centrifuged to give a crude PNA oligomer. It was further purified by reversed-phase HPLC on a Wakosil II 5-ODS-AR column (30×150 mm) using acetonitrile/water/TFA solvent system. The eluted product, which was detected by UV at 260 nm, was evaporated to remove water and TFA, and then the PNA oligomer was further collected by ether precipitation. Each PNA oligomer was characterized by MALDI-TOF MS (Table 1)[17] and UV spectra (Fig. 1). The yields of pure PNA oligomers were moderate (40–60% from the MBHA resin), but this synthetic procedure is suitable for the preparation of a PNA oligomer in large amounts.

The thermal stability of the hybrids of flavin-tethered PNA (**FPNA**) with the complementary DNA oligomer was examined by measuring their melting temperatures (Table 1). **FPNA3** containing a flavin moiety at the C terminal was slightly more unstable than **PNA1**, while **FPNA2** containing a flavin incorporated in the interior of the hybrid was less stable than **PNA1**. In addition, **FPNA1** containing the flavin moiety near the amino terminal region slightly stabilized the PNA–DNA hybrid. These results suggested that the flavin moiety, which was introduced near the amino terminal region rather than the C-terminal or the middle of the PNA oligomer, can contribute to the stabilization of the PNA–DNA hybrid.

**Table 1.** MALDI-TOF MS[17] of **FPNA1**∼**3** and melting temperatures of PNA–DNA hybrids[a]

| | PNA | MALDI-TOF $(M+H)^+$ | | $T_m$ (°C) |
|---|---|---|---|---|
| | | Calcd | Found | |
| **PNA1** | H-(GC TCT TCC CGC)-$NH_2$ | 2907.79 | 2906.70 | 59.3 |
| **FPNA1** | H-(GC TCT TCC C**F**C)-$NH_2$ | 3012.92 | 3012.85 | 61.3 |
| **FPNA2** | H-(GC TCT **F**CC CGC)-$NH_2$ | 3037.44 | 3038.11 | 50.2 |
| **FPNA3** | H-(**F**C TCT TCC CGC)-$NH_2$ | 3012.92 | 3012.95 | 55.5 |

[a] Each FPNA was annealed with 5′-d(CG AGA AGG GCG)-3′ (50 μM, strand concentration) in sodium cacodylate buffer (50 μM, pH 7.0). The sample was graduately heated from 2 to 90°C in 1°C increments with 1 min equilibration. Bold **F** means flavin PNA unit.

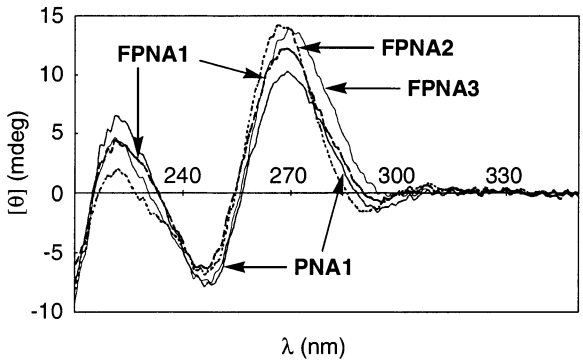


**Figure 2.** CD spectra of the hybrids of **PNA1** (—), **FPNA1** (···), **FPNA2** (– – –), and **FPNA3** (—) with 5′-d(CG AGA AGG GCG)-3′.

The CD spectra of the hybrids of **FPNA1-3** with complementary DNA oligomers showed that there was no significant difference between **PNA1** and **FPNA1-3** (Fig. 2). These results suggested that the hybrids of flavin-tethered PNA oligomers can serve as a functionalized antisense oligonucleotide.

In summary, we have synthesized for the first time flavin-tethered PNAs and examined their thermodynamic stability in hybridization with complementary DNA. Flavin-tethered PNA oligomers might be utilized as functionalized antisense molecules. We are also investigating whether flavin-conjugated PNA oligomers can serve as an artificial DNA photolyase for thymine dimer photosplitting.

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15. Spectral data for **5**: $^1$H NMR (400 MHz in $CD_3OD$): due to restricted rotation around the secondary amide, several of the signals were appeared as doubled; $\delta$ 8.01 (1 H, brs), 7.50 (mi) and 7.43 (ma) (1 H, brs), 5.63 (ma) and 5.15 (mi) (1 H, brs), 4.99 (ma) and 4.86 (mi) (2 H, brs), 4.25 (mi) and 4.09 (ma) (3 H, brs), 3.63 (ma) and 3.52 (mi) (2 H, brd), 3.47 (2 H, s), 3.39 (ma) and 3.27 (mi) (2 H, brd), 2.53 (3 H, s), 2.42 (3 H, s), 1.43 (6 H, s), 1.40 (3 H, s); HRMS ($FAB^+$, NBA/$CH_2Cl_2$) calcd for $C_{24}H_{31}O_7N_6$ [$(M+H)^+$] 515.2252, observed 515.2273; UV $\lambda_{max}$ (DMF) 390, 460 (nm).
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