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Synthesis and characterization of naphthalimide-containing peptide nucleic acid

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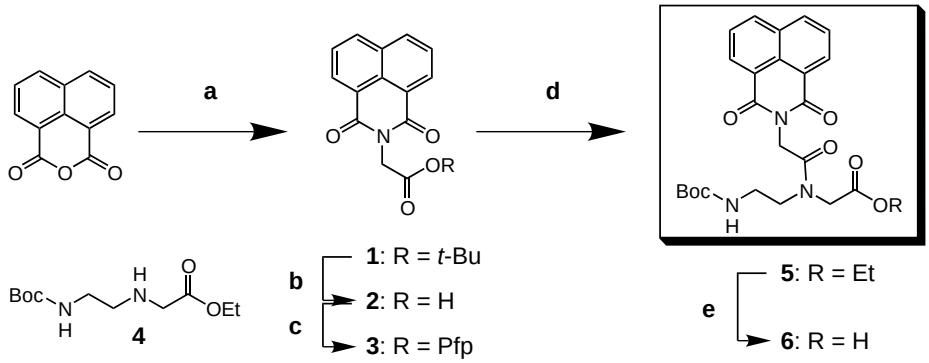
Abstract—Naphthalimide-containing PNA monomer unit **6** was synthesized for the preparation of PNAs containing a naphthalimide chromophore (**NPNA**). PNA oligomers were purified by reversed-phase HPLC and characterized by MALDI-TOF MS and UV spectra. Thermodynamic analyses revealed that the PNA oligomer containing a naphthalimide moiety at the amino terminal stabilizes PNA–DNA hybrids. © 2002 Elsevier Science Ltd. All rights reserved.

Peptide nucleic acid (PNA) is a DNA analogue in which the phosphate backbone is replaced by *N*-(2-aminoethyl)glycine units.[1] The electrostatically neutral peptide backbone prevents inter-strand repulsion of the complementary strand, allowing sequences to be recognized with high affinity and selectivity with respect to hybridization with DNA or RNA.[2] The use of PNAs is also advantageous because they are extremely stable to cellular nucleases and proteases.[3] The PNA oligomer can be easily synthesized by solid-phase *t*Boc/Fmoc peptide chemistry.[4] Several groups have carried out modifications of PNA backbones to improve the binding characteristics to DNA and RNA as well as to improve its membrane permeability.[5] Concurrently, synthesis of several functionalized PNA oligomers containing functionalized molecules in place of nucleobases have been reported to improve the water solubility and to study the electron transfer chemistry through DNA duplex.[6] Recently, we have demonstrated a strategic synthesis of a photosensitizable PNA monomer that enables the introduction of photoactive molecules into PNA oligomers at predetermined sites.[7]



Scheme 1. Preparation of naphthalimide-containing PNA monomer. *Reagents and conditions*: (a) *t*-butyl glycinate hydrochloride, triethylamine, xylene, reflux, 4 h, 87%; (b) HCOOH, rt, 2 h, 96%; (c) pentafluorophenol, DCC, DMF, rt, 4 h; (d) **4**, diisopropylethylamine, DMF, 10 h, 72% from **3**; (e) 2N aq. NaOH, EtOH, 2 h, then Dowex 50Wx8 (Py^+) treatment, 92%.

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Naphthalimides are well-known as potential anti-tumor agents as exemplified by mitonafide, amonafide, and azonafide.[8] Numerous naphthalimide analogues have been synthesized, and qualitative structure–activity relationships (QSARs) were studied to shed light on their intercalative inhibition of topoisomerase II.[9] Recently, related compounds, bis-naphthalimide LU 79553 and DMP-840, were reported to bisintercalate into DNA at the major groove and inhibit topoisomerase II.[10] Establishment of an effective method for introducing intercalating reagents such as naphthalimide derivatives at specific oligomer sites is particularly useful for studying PNA–DNA hybrid stability aimed at the development of antisense oligonucleotides. The protocol for the preparation of PNAs containing a hybrid stabilizer at the desired sites has not been described. Here we report the preparation of a naphthalimide-containing PNA monomer and the characterization of naphthalimide-containing PNA oligomers.

Naphthalimide-containing PNA monomer **6** was synthesized as shown in Scheme 1. A mixture of 1,8-naphthalic anhydride and *t*-butyl ester of glycine was refluxed in xylene, evaporated, and recrystallized from EtOH to give **1** (87%). Ester **1** was acid-hydrolyzed and the residue was flash-chromatographed to give **2** (96%). Treatment of **2** with pentafluorophenol and DCC followed by condensation with ethyl *N*-(2-((*t*-butoxy)carbonylamino)ethyl)glycinate[11] (**4**) and diisopropylethylamine gave **5** (72% from **2**). Ester **5**, which was hydrolyzed by 2N aq. NaOH, was directly flash-chromatographed on DOWEX 50Wx8 (pyridinium form) column, evaporated, and recrystallized with EtOH to give **6** (92%). It was possible to monitor the incorporation of the naphthalimide chromophore into PNA oligomers by simply measuring UV spectra having a UV absorption at ca. 340 nm which was not overlapped with PNA nucleobases, as shown in Fig. 2.

Several PNA oligomers were synthesized by solid-phase *t*Boc chemistry as previously described.[12] A Ninhydrin test showed that each coupling of PNA monomers with the MBHA resin using HBTU was satisfactory. HPLC analysis showed that the crude PNA oligomers could be effectively prepared together with only a small amount of shorter PNA oligomers as by-products (Fig. 1). Each purified PNA oligomer was characterized by MALDI-TOF MS (Table 1) and UV spectra (Fig. 1). UV absorptions of **NPNA1**–**2** at 348 nm indicated that naphthalimides had been introduced into PNA oligomers. The intensity at 348 nm of **NPNA2** was about twice that of **NPNA1**, indicating that **NPNA2** has two naphthalimide moieties in a PNA oligomer. However, the intensity of **NPNA7** was less than three times that of **NPNA1**, suggesting that any hypochromicity caused by the three consecutively incorporated naphthalimide moieties might be indicative of the restricted space within the interior region of **NPNA7**.

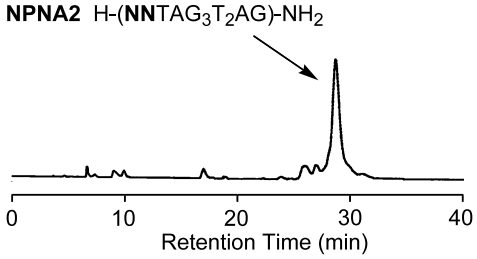

Figure 1. HPLC profile of the crude **NPNA2**. Each crude PNA oligomer was purified by reversed-phase HPLC on a Wakosil II 5-ODS-AR column (30×150 mm) using acetonitrile/water/0.1% TFA solvents system. Bold **N** means naphthalimide PNA unit.

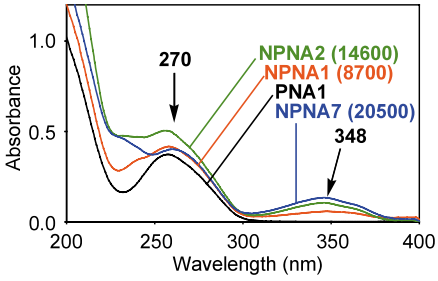

Figure 2. UV spectra of **PNA1**, **NPNA1**, **2**, and **7**. Each sample (5 μM, strand concentration) dissolved in water/CH_3CN (1:1) was measured in 10 mm quartz cuvette on a UV/VIS spectrophotometer. Each molar extinction coefficient (ε, cm^2/mmol) at 348 nm was inserted in parenthesis.

Table 1. MALDI-TOF MS and melting temperatures[a] of naphthalimide-containing PNA **NPNA1**–**7**

		MALDI-TOF MS		Melting temperature[a]	
		Calcd	Found	T_m (°C)	ΔT_m (°C)
H-p(G TTA GGG TTA G)-NH_2	**PNA1**	3088.89	3088.51	64.5	
H-p(**N** TTA GGG TTA G)-NH_2	**NPNA1**	3134.96	3134.52	65.0	0.5
H-p(**N** NTA GGG TTA G)-NH_2	**NPNA2**	3206.03	3206.21	67.8	3.3
H-p(G TTA GGG TTA **N**)-NH_2	**NPNA3**	3134.96	3135.50	56.7	−7.8
H-p(G TTA GGG TTN **N**)-NH_2	**NPNA4**	3197.02	3196.88	60.3	−4.2
H-p(G TTA GNG TTA G)-NH_2	**NPNA5**	3134.96	3135.31	35.2	−29.3
H-p(G TTA **N**NG TTA G)-NH_2	**NPNA6**	3181.02	3180.98	27.5	−37.0
H-p(G TTA **NNN** TTA G)-NH_2	**NPNA7**	3227.08	3227.11	N.D.	N.D.

[a] Each sample was heated from 20.0 to 90.0°C in 1.0°C increments with 1 min equilibration. Concentration of PNA–DNA, 5′-d(C TAA CCC TAA C)-3′, hybrid was 5 μM (each strand) in 50 mM cacodylate buffer (pH 7.0) and 100 mM Na^+. Bold **N** means naphthalimide PNA unit.

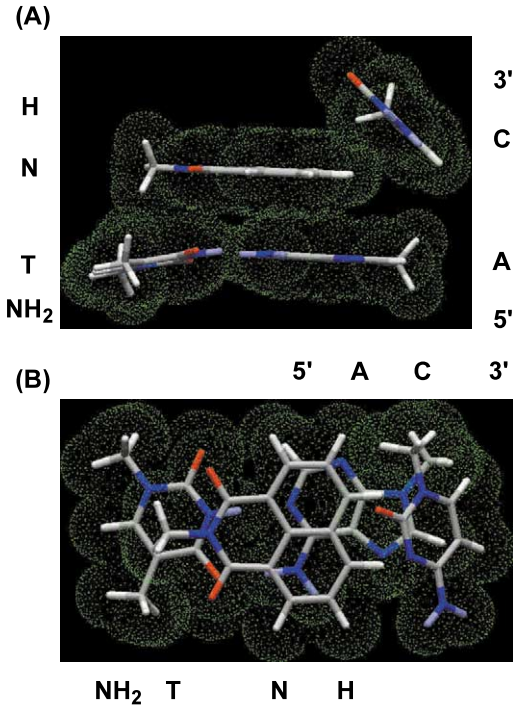

Figure 3. Molecular models of the **NPNA1** and 5′-d(CG AGA AGG GCG)-3′ hybrid. (A) Horizontal view of [p(**NT**)/d(AC)] region; (B) vertical view of [p(**NT**)/d(AC)] region. These models were obtained through optimization of the hybrid of **NPNA1** and 5′-d(CG AGA AGG GCG)-3′ by AMBER* force field in water set by using MacroModel version 6.0. After the energy minimization, other base pairs were removed. Bold **N**, **T**, **C**, and **A** mean naphthalimide PNA, thymine PNA, deoxycytidine, and deoxyadenosine, respectively. van der Waals surface is shown in dotted green.

The thermal stability of the hybrids of naphthalimide-containing PNA oligomers (**NPNA1**–**7**) with 5′-d(CTAA CCC TAA C)-3′ was examined by measuring melting temperatures (T_m, Table 1). **NPNA1** containing a naphthalimide moiety at the amino terminal of the PNA oligomer was slightly more stable than **PNA1**. **NPNA2** containing two neighboring naphthalimide moieties at the amino terminal was considerably more stable than **PNA1**, even though there were fewer base pairs in the **NPNA2**/5′-d(C TAA CCC TAA C)-3′ hybrid compared with **PNA1**. Although **NPNA3**–**4** containing naphthalimide moieties at the C-terminal was less stable than **PNA1**, the stabilization effect of the PNA–DNA hybrid by the two neighboring naphthalimide moieties was evident. In contrast, **NPNA5**–**7** containing naphthalimide moieties incorporated in the interior of the PNA–DNA hybrids was significantly less stable than **PNA1**. In particular, **NPNA7** containing three consecutive naphthalimide moieties could not hybridize with 5′-d(C TAA CCC TAA C)-3′, indicating that the introduction of naphthalimide moieties into the interior of the PNA oligomer is not suitable for the stability of the PNA–DNA hybrid as a consequence of the steric hindrance of the naphthalimide skeleton. These results suggested that the naphthalimide moiety introduced at the amino terminal region rather than the C-terminal or the middle of the PNA oligomer contributes to the stabilization of the PNA–DNA hybrid. These results are in agreement with the thermal stability of the flavin-containing PNA oligomers previously reported.[7a]

In order to investigate the basis for PNA–DNA hybrid stabilization, we carried out molecular mechanics (MM) calculations of the PNA–DNA hybrids using AMBER* force field as previously described.[13] Minimization energy of the **NPNA1**–DNA hybrid (−3398.42 kcal/mol) and **NPNA2**–DNA (−3371.22 kcal/mol) hybrid was smaller than that of the **PNA1**–DNA hybrid (−3161.12 kcal/mol); nevertheless, a naphthalimide moiety pushed out the cytosine as a base pair partner (Fig. 3A). The π–π interaction by the naphthalimide moiety extended to near adenine with 5′-d(C TAA CCC TAA C)-3′ in addition to the neighboring thymine in the PNA oligomer (Fig. 3B). In **NPNA2**, two adjacent naphthalimides were strongly stacked with a slight spread such as a folding fan, suggesting that the naphthalimides could effectively form the π–π interaction with the neighbor base pairs and an unpaired adenine base (data not shown). These results suggested that the naphthalimide moiety played an important role in the stabilization of the PNA–DNA hybrid by the extended π–π interaction.

We also believe that the introduction of a naphthalimide moiety at the amino terminal of the PNA oligomer promotes the stability of the PNA–DNA hybrids as the terminal amino group is electrostatically attracted to the nearest neighboring irregular phosphate of the DNA oligomer.

In summary, we have synthesized naphthalimide-containing PNA oligomers for the first time and demonstrated the thermodynamic stability in hybridization with the corresponding DNA oligomer. We believe that naphthalimide-containing PNA oligomers would be attractive as a potent anti-tumor agent and antisense molecule.

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