Peptide Nucleic Acid with a Lysine Side Chain at the \( \beta \)-Position: 
Synthesis and Application for DNA Cleavage

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This paper reports the synthesis of new \( \beta \)-Lys peptide nucleic acid (PNA) monomers and their incorporation into a 10-residue PNA sequence. PNA containing \( \beta \)-Lys PNA units formed a stable hybrid duplex with DNA. However, incorporation of \( \beta \)-Lys PNA units caused destabilization of PNA–DNA duplexes to some extent. Electrostatic attractions between \( \beta \)-PNA and DNA could reduce this destabilization effect. Subsequently, bipyridine-conjugated \( \beta \)-Lys PNA was prepared and exhibited sequence selective cleavage of DNA. Based on the structures of the cleavage products and molecular modeling, we reasoned that bipyridine moiety locates within the minor groove of the PNA–DNA duplexes. The lysine side chain of \( \beta \)-PNA is a versatile handle for attaching various functional molecules.

Key words  
peptide nucleic acid (PNA); chirality; duplex stability; DNA recognition; DNA cleavage

Peptide nucleic acid (PNA) is a synthetic analogue of DNA in which the sugar-phosphate backbone of DNA is replaced by an artificial peptide backbone consisting of N-(2-aminoethyl)-glycine units. PNAs hybridize to complementary sequences by the Watson–Crick base pairing rule with superior thermal stability compared to natural DNA/RNA. This is because the neutral PNA backbone does not have the electrostatic repulsion that destabilizes DNA–DNA duplexes. The relatively rigid PNA backbone improves sequence selectivity on hybridization. PNAs are resistant to nucleases and proteases due to their unnatural backbone, and are unlikely to bind to proteins. These unique properties make PNAs an attractive agent for next-generation nucleic acid-based therapeutics. To improve the antisense and antigene properties of PNAs, a lot of PNA analogues have been reported, some of them possessing improved DNA/RNA binding properties.

An approach for tailoring the hybridization behavior of PNA is to introduce substituents to the PNA backbone. Several chiral PNAs with substituents at the \( \alpha \), \( \beta \), or \( \gamma \) positions have been reported. The effects of substituents at the \( \alpha \)- or the \( \gamma \)-position have been extensively studied and revealed that \( \gamma \)-substitution is more effective to improve the DNA binding properties of PNAs than \( \alpha \)-substitution. We recently reported a chiral PNA with a methyl group at the \( \beta \)-position (\( \beta \)-Me PNA) and showed that the stereochemistry of the \( \beta \)-carbon was critical to the hybridization ability of PNA and was strictly limited to the \( S \)-configuration. Incorporation of the \( R \)-configuration monomer into PNA oligomers was detrimental to hybridization with DNA. While the introduction of a methyl group at the \( \beta \)-position preorganized the single-stranded PNA oligomer into a right-handed helical structure, the DNA binding ability of the \( \beta \)-Me PNA was slightly lower than that of unmodified PNA. To improve the DNA binding properties of \( \beta \)-PNA, we planned to introduce a lysine side chain at the \( \beta \)-position, expecting electrostatic attraction between a positively charged amino group of the PNA side chain and negatively charged phosphate of the DNA backbone.

Results and Discussion

Design and Synthesis of \( \beta \)-Lys PNA Monomers 
We decided to introduce the L-lysine side chain at the \( \beta \)-position of the PNA backbone because our previous study revealed that \( \beta \)-Me PNA, in its \( S \)-configuration only, was suited for hybridizing to the complementary DNA sequence (Fig. 1). We also anticipated that the lysine side chain would be a versatile handle for attaching a variety of functional molecules without disrupting the DNA binding ability of PNAs as in the case of \( \gamma \)-PNA.

Chart 1 illustrates the synthesis of chiral \( \beta \)-Lys PNA

**Fig. 1.** Structures of A) PNA, B) \( \beta \)-(S)-Me-PNA, and C) \( \beta \)-(S)-Lys PNA

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monomers 7 and 8. In each monomer, the amino group of the backbone was masked as an azide that can be reduced under mild conditions and should be suited for coupling with the next PNA monomer on the solid support. The monomers were synthesized from the commercially-available Fmoc, Boc-protected l-lysinol derivative 1 derived from l-lysine. Conversion of the amino alcohol 1 to the iodide 2 using I$_2$, PPh$_3$ and imidazole (99%) followed by a displacement with NaN$_3$ afforded the azide 3 in 63% yield. The Fmoc group of 3 was removed by alkaline treatment (99%) and the resulting amine 4 was alkylated with methyl bromoacetate in CH$_2$Cl$_2$ to give the protected β-Lys PNA backbone 5 in 64% yield. Coupling of the thymin-1-ylacetic acid was accomplished with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) in N,N-dimethylformamide (DMF) to afford 6 in quantitative yield. Alkaline hydrolysis of the methyl ester 6 proceeded quantitatively to give β-Lys PNA monomer 7. In addition, in order to introduce functional molecules onto β-Lys PNA during solid-phase synthesis of the PNA oligomer, the Boc group of the side chain was converted to the Fmoc group that can be deprotected without affecting the azide group of the backbone. The monomer 7 was treated with 4 M HCl to remove the Boc group followed by Fmoc protection with FmocOSu to give orthogonally-protected β-Lys PNA monomer 8 in 97% yield for two steps from 7.

Synthesis of PNA Oligomers To evaluate the effect of a lysine side chain at the β-position on DNA binding of PNA, one or two units of the β-Lys PNA monomer 7 were incorpo-
rated into PNA oligomers using a standard manual solid-phase peptide synthesis procedure (Table 1, P4, P5). A 10-residue mixed-base PNA, P1, was used as a reference because this sequence has been well studied. During the course of solid-phase synthesis, the azide group was reduced by 5 min of treatment with trimethylphosphine. After deprotection and cleavage from the resin by TFA treatment, each PNA was purified by reversed-phase HPLC and characterized by matrix-assisted laser desorption/ionization (MALDI)-orbitrap or electrospray ionization time-of-flight (ESI-TOF) mass spectrometry. The synthesized PNA oligomers are listed in Table 1 (sequences) and Table 2 (mass results).

Hybridization Studies of PNAs with Complementary DNA To evaluate the effect of β-Lys backbone on the DNA binding ability of PNA, the melting temperatures ($T_m$) of duplexes with complementary DNA in 10 mM sodium phosphate buffer containing 1 mM ethylenediaminetetraacetic acid (EDTA) were measured. The UV melting profiles of β-Lys PNA–DNA duplexes exhibited helix-to-coil transitions (Fig. 2). The $T_m$ values were determined from the first derivatives of the UV-melting curves and are shown in Table 3. Although the cationic amino groups of β-Lys PNAs were expected to improve the thermal stability of PNA–DNA duplexes, the modified PNA with a single β-Lys PNA unit, P4, had a lower $T_m$ value compared to unmodified PNA P1. The PNA–DNA duplex was further destabilized when two β-Lys PNA units were incorporated (Table 3, P5). The destabilization effect of β-Lys PNA units was relatively small at lower salt concentrations. For example, P4 was destabilized by 6.4°C at 1000 mM NaCl compared to P1. However, destabilization of P4 was only 1.4°C at 0 mM NaCl. This is because the electrostatic attractions between cationic PNA side chains and negatively charged phosphates of DNA were more effective at lower salt concentrations and partially compensated for the influence of steric hindrance of the side chains. We have recently reported the PNA containing three $S$-form β-Me PNA units (named PNA 2 in ref. 12, H-GTATGCAC-Lys-NH$_2$, $T_\beta$-Me PNA) whose $T_m$ value was 51°C at 0 mM NaCl. Although the methyl group of β-Me PNA induced a right-handed helical structure, the induced structure did not contribute to the total hybridization stability. This is because the benefit of the helical structure was canceled by the steric hindrance arising from the methyl group. The aminobutyl group of β-Lys PNA is significantly larger than the methyl group of β-Me PNA and a marked destabilization relative to PNA 2 was anticipated. In fact, the PNA–DNA duplexes formed by P4 or P5 were less stable than that of PNA 2. However, the destabilization was small ($\Delta T_m$=1°C for P4, $\Delta T_m$=1.6°C for P5). Such a small difference can be explained by the electrostatic effect of positively charged lysine side chains.

We compared the difference in the $T_m$ values between fully

<table>
<thead>
<tr>
<th>Table 2. Mass Results of PNA Oligomers</th>
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<tbody>
<tr>
<td><strong>Calc</strong></td>
</tr>
<tr>
<td>P4</td>
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<tr>
<td>P5</td>
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<tr>
<td>P6</td>
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<tr>
<td>P7</td>
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<td>P8</td>
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| | a) ESI-TOF-MS. b) MALDI orbitrap. |

Table 3. $T_m$ (°C) Values of the Hybrid Duplexes of PNA–DNA$^{(a)}$

<table>
<thead>
<tr>
<th>[NaCl]</th>
<th>0 mM</th>
<th>100 mM</th>
<th>1000 mM</th>
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<tbody>
<tr>
<td>P1</td>
<td>51.4$^{(b)}$</td>
<td>49.7</td>
<td>45.6</td>
</tr>
<tr>
<td>P4</td>
<td>50.0</td>
<td>44.1</td>
<td>39.2</td>
</tr>
<tr>
<td>P5</td>
<td>49.4</td>
<td>41.5</td>
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</table>

Table 4. $T_m$ (°C) Values of the Mismatched Duplexes of PNA–DNA$^{(a)}$

<table>
<thead>
<tr>
<th></th>
<th>Fullmatch DNA$^{(b)}$</th>
<th>Mismatch DNA$^{(c)}$</th>
<th>$\Delta T_m$ (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>49.7</td>
<td>34.6</td>
<td>15.1</td>
</tr>
<tr>
<td>P4</td>
<td>44.1</td>
<td>24.2</td>
<td>19.9</td>
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<table>
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<tr>
<th></th>
<th>PO1</th>
<th>PO4</th>
<th>PO5</th>
</tr>
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<tbody>
<tr>
<td>A</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td><img src="image2.png" alt="Normalized Absorbance" /></td>
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</table>

All samples were prepared in 10 mM sodium phosphate buffer containing 1 mM EDTA (pH 7.4). Thermal denaturation was monitored at 260 nm at a rate of 0.2°C per min A) 0 mM NaCl and B) 100 mM NaCl.
matched PNA–DNA duplexes and the duplexes containing a single G-T mismatch sequence. As shown in Table 4, the \( \beta \)-PNA P4 exhibited superior sequence selectivity compared to unmodified PNA P1. This is a positive effect of steric hindrance of the lysine side chain.

**Sequence-Specific DNA Cleavage by \( \beta \)-PNA Bearing Bipyridine at the Side Chain** To demonstrate the lysine side chain at the \( \beta \)-position as a versatile handle, we chose 2,2'-bi-bipyridine as a functional molecule to be attached because bipyridine is known to induce DNA damage in the presence of copper ion under physiological conditions. Since 1,10-phenanthroline cleaves DNA in nuclei by using endogenous copper ions,19 the same mechanism can be expected for bipyridine in living cells because of the structural similarity. With this in mind, we attached bipyridine at the lysine side chain of \( \beta \)-PNA (Table 1, P6). Bipyridine unit 12 was prepared as depicted in Chart 2.

P6 was synthesized on the solid support using \( \beta \)-Lys PNA monomer 8. After the coupling of monomer 8, the Fmoc group of the side chain was removed by piperidine treatment followed by coupling with Boc-protected bipyridine unit 12 using 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridine-1-ium 3-oxide·hexafluorophosphate (HATU) as a coupling agent. The subsequent procedure was the same as described in the section of PNA oligomer synthesis. To evaluate the effect of the position at which bipyridine was attached on DNA cleavage, we additionally prepared two conjugates in which bipyridine was linked to a 12-residue mixed-base PNA oligomer at the N-terminus (Table 1, P7, P8). The difference between P7 and P8 was the linker length.

**DNA Cleavage by PNAs** DNA cleavage activity of P6 was tested on a 54-mer DNA substrate D1 containing a 17 bp hairpin structure for ethidium staining. The DNA substrate for P7 and P8 was a 51-mer DNA oligomer D2 containing the same hairpin structure (Fig. 3C). Cleavage reactions were carried out by incubating the DNA substrates with the PNA

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**Chart 2. Synthesis of Boc-Protected Bipyridine Unit**

![Chart 2](chart2.png)

**Fig. 3. PAGE Analysis of DNA Cleavage by (A) P6 or (B) P7 or P8. (C) DNA Substrates Used**

![Fig. 3](fig3.png)
conjugates at 4°C for 24 h in the presence of CuSO₄ and mercaptaptopropionic acid (MPA). Reaction products were analyzed by denaturing polyacrylamide gel electrophoresis (Figs. 3A, B). **P6** exhibited a specific DNA cleavage activity (Fig. 3A, lane 4). In contrast, neither **P7** nor **P8** displayed significant DNA cleavage activities (Fig. 3B, lanes 6, 7). These results demonstrated that efficient DNA cleavage was observed only when the bipyridine unit was attached to the side chain of the β-PNA unit placed at the center of the PNA oligomer. The cleavage yield with **P6** on the DNA substrate **D1** was 27%. No specific cleavage was observed when the pipyridine was linked to the N-terminus of the PNA irrespective of linker structure and length. In the PNA–DNA complex formed by **P7** or **P8** with the DNA substrate **D2**, the bipyridine moiety should locate close to the DNA sequence of 5'-TCTAC-3'. Since this sequence was cleaved in β-PNA–DNA duplex (Fig. 3A), the failure of DNA cleavage by bipyridine linked to the N-terminus of PNA would not be due to the DNA sequence but was probably due to the flexibility of single-stranded region of the DNA substrate.

The major products of the sequence selective cleavage by **P6** were analyzed by MALDI-TOF mass spectrometry. To simplify the product analysis, a 10-mer DNA oligonucleotide **D3** (5'-AGTGATCTAC-3') was used as a substrate for cleavage by **P6**. We assign the cleavage product at *m/z* = 1212.4 as a 4-mer DNA with a 5'-phosphate terminus and the product at *m/z* = 1605.5 as a 5-mer DNA with a 3'-phosphate terminus, respectively (Fig. 4). DNA fragments with a terminal phosphate are the most common products observed with oxidative cleavage.20,21) Thus, these two major products indicate that **P6** cleaved the 10-mer DNA through oxidative destruction of the deoxythymidine that is the fifth residue from the 3'-terminus. By linking bipyridine to the side chain of β-Lys PNA, the metal-binding ligand was forced to project along the minor groove passing close to hydrogen atoms that can be abstracted. The bipyridine can reach the deoxyribose of the deoxythymidine directly below the deoxyadenine paired to the thymine of β-Lys PNA in the 3'-direction. Molecular modeling also supported hydrogen abstraction from the deoxythymidine in the minor groove (Fig. 5).

In summary, we synthesized two new β-Lys PNA monomers, 7 and 8, and incorporated them into a 10-mer PNA sequence. PNA containing the β-Lys PNA units formed a stable hybrid duplex with complementary DNA. However, incorporation of β-Lys PNA units destabilized PNA–DNA duplexes more than unmodified PNA did. Electrostatic interactions between cationic lysine side chains of β-PNA and negatively charged phosphates of DNA can reduce the destabilization effect caused by steric hindrance of the side chains. The bipyridine-conjugated β-Lys PNA sequence selectively cleaved DNA. When bipyridine was appended at the N-terminus of an unmodified PNA, no specific cleavage was observed. Based on the structures of the cleavage products and molecular modeling, we reasoned that bipyridine moiety was located within the minor groove of the PNA–DNA duplexes and cleaved DNA efficiently. The lysine side chain of β-PNA is a versatile handle for attaching various functional molecules. We hope that the β-PNAs reported here will expand the scope of application of PNA s in the areas of therapeutics and nanotechnologies.

**Experimental**

1H- and 13C-NMR spectra were recorded on JEOL JNM-ECS 400 NMR (400 MHz) spectrometer. 1H-NMR spectra were referenced with (CH₃)₄Si (δ 0.00 ppm) as an internal standard. 13C-NMR spectra were referenced with deuterated solvent (δ 77.0 ppm for CDCl₃). High resolution (HR)-MS were obtained on an LCMS-IT-TOF mass spectrometer (Shimadzu) in ESI method or MALDI DUO orbitrapXL mass spectrometer (Thermofisher Scientific). Optical rotations were measured on a JASCO-P-2200 digital polarimeter. Column chromatography was performed on silica gel 60N (Kanto Chemical Co., Inc., 100–210µm). HPLC was carried out on a Hitachi HPLC system consisting of the following equipment: pump, LC-2130; detector, L-2400; column, Mightysil RP-8 GP (250 mm×4.6mm, 5µm) for analysis and Wakopak Navi C18-5 (250 mm×10mm) for purification. All experiments were performed under anhydrous conditions in an atmosphere of argon, unless otherwise mentioned.

(9H-Fluoren-9-yl)methyl tert-Butyl(6-azidohexane-1,5-diyi)-(S)-dícárba-mate (3) Compound 3 was prepared according to the literature27; 1H-NMR (400MHz, CDCl₃) δ: 1.43 (s, 9H), 1.33–1.53 (m, 6H), 3.07–3.14 (m, 2H), 3.34–3.45 (m, 2H), 3.72–3.79 (m, 1H), 4.20–4.23 (m, 1H), 4.37–4.47 (m, 2H), 4.57 (s, 1H), 4.91 (s, 1H), 7.31 (t, *J*=7.4Hz, 2H), 7.40 (t, *J*=7.4Hz, 2H), 7.59 (d, *J*=7.3Hz, 2H), 7.76 (d, *J*=7.6Hz, 2H); HR-MS (ESI) Calcd for C₂₇H₂₅N₂O₂Na [M+Na]⁺ 502.2425. Found 502.2429.

Methyl (5)-[1-Azido-6-(trans-butoxycarbonyl)aminohexa-2-ylglycinato] (5) To a solution of 3 (802.7 mg, 1.67 mmol) in MeOH (30 mL) was added 2 M NaOH (6 mL, 12 mmol) at 0°C with stirring at room temperature (r.t.) for 1.5 h. H₂O (15 mL) was added and the pH was adjusted to 4 by addition of 2 M HCl followed by evaporation of MeOH. After addition of H₂O (50 mL), the aqueous phase was washed with CH₂Cl₂.
(2×30 mL). The aqueous phase was then made strongly alkaline by addition of 2 M NaOH and extracted with CH₂Cl₂ (4×30 mL). The organic phase was dried over Na₂SO₄, filtered and concentrated in vacuo to afford 4 (429.6 mg, 1.67 mmol, quant.) as a colorless oil. To a solution of 4 (429.6 mg, 1.67 mmol) in CH₂Cl₂ (30 mL) was added EDCI (689 mg, 3.59 mmol) at 0°C under argon. The solution was stirred at r.t. for 8 d. After addition of H₂O (5 mL), the mixture was extracted with EtOAc (30 mL). The organic phase was dried over Na₂SO₄, filtered and concentrated in vacuo. The residue was purified by column chromatography on silica gel (CH₂Cl₂:MeOH = 1:99:1–1:9:1) to give 6 (93.4 mg, 98%) as a white powder; 1H-NMR (400 MHz, CDCl₃) δ: 1.45 (s, 9H), 3.09–3.13 (m, 2H), 3.27–3.32 (m, 2H), 4.15–4.28 (m, 2H), 4.50–4.58 (m, 1.5H), 4.84 and 4.87 (each s, total 1H), 7.07 (d, J = 1.1 Hz, 1H), 9.60 (s, 1H); HR-MS (ESI) Calcd for C₁₇H₁₅N₃O₄Na [M⁺Na⁹⁺] 518.2334. Found 518.2330.

Methyl (S)-N-[1-Azido-6-[1-(tert-butoxycarbonyl)amino]hexan-2-yl]-N-[2-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-2(1H)-yl)acetylglycinate (6) To a solution of 5 (592 mg, 1.80 mmol) and thymin-1-ylactic acid (661.9 mg, 3.59 mmol) in DMF (15 mL) was added EDCI (689 mg, 3.59 mmol) at 0°C under argon. The solution was stirred at r.t. for 18 h. After addition of H₂O (60 mL), the mixture was extracted with EtOAc (30 mL×3), washed with 1 M HCl, H₂O, saturated NaHCO₃, H₂O, and brine, dried over Na₂SO₄, filtered and concentrated in vacuo. The residue was purified by column chromatography on silica gel (hexane:EtOAc = 1:1 to give 6 (930.1 mg, 1.80 mmol, quant.) as a viscous oil; 1H-NMR (600 MHz, CDCl₃) δ: 1.18 (m, 2H), 1.26–1.51 (m, 6H), 1.80 and 1.86 (each s, total 3H), 3.03–3.17 (m, 2H), 3.37–3.55 (m, 2H), 3.86–3.93 (m, 1H), 4.09–4.69 (m, 7H), 5.20 and 5.41 (each t, J = 5.5 Hz), 7.29 (t, J = 7.1 Hz, 2H), 7.37 (t, J = 7.1 Hz, 2H), 7.55–7.59 (m, 2H), 7.73 (d, J = 6.9 Hz, 2H), 9.75 and 9.93 (each s); HR-MS (ESI) Calcd for C₁₃H₁₅N₃O₄Na [M⁺Na⁹⁺]: 362.2334. Found 362.2319.

Ethyl 5’-(2-(1-tert-Butoxy carbonyl)amino)ethyl)carbamo)-[2,2'-bipyridine]-5-carboxylate (11) To a solution of 9(22) (207 mg, 0.76 mmol) and 10 (181 µL, 1.14 mmol) in DMF (10 mL) were added EDCI (218.5 mg, 1.14 mmol), N,N-dimethyl-4-aminopyridine (DMAP) (28 mg, 0.23 mmol), and Et₃N (159 µL, 1.14 mmol) at 0°C with stirring at r.t. for 41 h. The reaction mixture was poured into 1 M HCl (50 mL) and extracted with EtOAc (2×40 mL), washed with H₂O, sat. NaHCO₃, H₂O, and brine, dried over Na₂SO₄, filtered and concentrated in vacuo. The residue was purified by column chromatography on silica gel (CH₂Cl₂:MeOH = 1:99:1) to give 11 (93.4 mg, 98%) as a white powder; 1H-NMR (400 MHz, CDCl₃) δ: 1.45 (s, 12H), 3.43–3.47 (m, 2H), 3.59–3.63 (m, 2H), 4.45 (q, J = 7.1 Hz, 2H), 5.03 (s, 1H), 7.65 (s, 1H), 8.27 (dd, J = 8.2, 2.1 Hz, 1H), 8.43 (dd, J = 8.3, 2.0 Hz, 1H), 8.56 (dd, J = 8.3, 4.2 Hz, 1H), 9.16 (s, 1H), 9.29 (d, J = 1.5 Hz, 1H); 13C-NMR (100 MHz, CDCl₃) δ: 14.3, 28.7, 78.2, 121.2, 121.4, 126.3, 129.8, 135.6, 137.9, 148.2, 150.4, 158.7, 157.8, 165.1, 165.5; HR-MS (ESI) Calcd for C₂₁H₂₇N₄O₅ [M⁺] + : 415.1976. Found 415.1971.

5’-(2-(1-tert-Butoxy carbonyl)amino)[ethyl]carbamo)-[2,2'-bipyridine]-5-carboxylic Acid (12) To a solution of 11 (161.5 mg, 0.39 mmol) in CH₂Cl₂ (3 mL) and MeOH (4 mL) was added 2 M NaOH (1 mL, 2 mmol) with stirring at r.t. for 1 h. H₂O (5 mL) was added and the pH was adjusted to 3 by addition of 1 M HCl. The mixture was extracted with CH₂Cl₂–MeOH (1:1) to give 12 (147.1 mg, 0.38 mmol, 98%) as a white powder; 1H-NMR (400 MHz, d₆-DMSO) δ: 1.31 (s, 9H), 3.09–3.13 (m, 1H), 3.27–3.32 (m, 2H), 6.91–6.94 (m, 1H), 8.33–8.39 (m, 2H), 8.45–8.50 (m, 2H), 8.82–8.85 (m, 1H), 9.10 (s, 1H), 9.13 (d, J = 1.3 Hz, 1H); 13C-NMR (100 MHz, d₆-DMSO) δ: 28.7, 78.2, 121.2, 121.4, 127.5, 131.0, 137.0, 138.9, 149.1, 150.9, 156.3, 158.0, 165.0, 166.6; HR-MS (ESI) Calcd for C₁₉H₂₃N₄O₆ [M⁺H⁺] + : 387.1663. Found 387.1650.

**Oligomer Synthesis** PNA and β-PNA oligomers were manually synthesized from β-Lys PNA monomers, unmodified PNA monomers, and β-Fmoc-Ne-Boc-lysines using the standard Fmoc-protected solid-phase synthesis protocol. Fmoc-PAL-PEG-PS resins were used as a solid support. HATU, N,N-diisopropylethylamine and 2,6-lutidine were used for coupling each monomer. Completion of the coupling reaction was monitored by the Kaiser test. Twenty percent piperidine in DMF was used for deprotection of the Fmoc-protecting group from the terminal amino group. The unreacted amines were capped with acetic anhydride. After coupling the last
monomer, the resins were treated with TFA containing 13.9% m-cresol and 2.8% H₂O to cleave the oligomers from the resins as well as to remove the protecting groups from the nucleobases and amino groups (Boc). The solvent was removed, and the residue was washed with Et₂O three times, dissolved in H₂O, and purified by reversed-phase HPLC.

**Azide Reduction-Coupling** The resin was treated with a 0.9 M PMe₃ in THF–H₂O (9:1; 1 mL) for 5 min then washed with NMP (3×1 mL). Subsequent coupling was performed with double coupling.

**Attachment of Bipyridine Unit 12 onto β-PNA** Piperidine (20%) in DMF was used for deprotection of the Fmoc-protecting group from the side chain amino group. Subsequent coupling of Boc-protected bipyridine unit 12 was performed with double coupling using HATU as a coupling agent.

**Thermal Denaturation Analysis** All PNA stock solutions were prepared in Milli-Q water. The concentrations of the PNA oligomers were determined at 85°C using the molar extinction coefficients of the corresponding nucleobases (T=8.6 cm²/μmol; C=6.6 cm²/μmol; G=11.7 cm²/μmol and A=13.7 cm²/μmol).

Prior to the experiment, all buffers were degassed by heating to 90°C for 10 min. The samples were prepared in 10 mM sodium phosphate buffer containing 1 mM EDTA (pH 7.4) and the indicated concentrations of NaCl. The concentration of each strand was 5 μM. All samples were annealed by heating at 90°C for 10 min followed by a gradual cooling to r.t. Thermal denaturation was monitored at 260 nm at a rate of 0.2°C/min. The Tₘ values were determined from the first derivatives of the UV-melting curves.

**Electrophoretic Mobility Shift Assays of DNA Cleavage** The DNA cleavage reactions by PNA were studied by use of electrophoretic mobility shift assays. Cleavage reactions were prepared by mixing 1 μL of 100 mM phosphate buffer pH 7.4, 1 μL of 20 μM DNA and 2 μL of 10 μM PNA with water to a total volume of 8 μL.

**D1:**
DNA sequence for P6
5'-TTTTTTTTTTTTAGTTTCCTACC GGCTGATCGATTCTGACTGGAATTCGATCGCGGCGG3' -3'  
**D2:**
DNA for P7 and P8
5'-AGCTAAACGTGTTAGCTTCCGGC TGATCGATTCTGACTGGAATTCGATCGCGGCGG3'.

Solutions were heated at 95°C for 5 min and then slowly cooled to 4°C. Cleavage reaction was initiated by addition of 1 μL of 100 μM CuSO₄ and 1 μL of 30 mM MPA (3-mercaptopropionic acid). Reactions were incubated at 4°C for 24 h and quenched by addition of 1 μL of 20 mM EDTA. Reaction products were separated and quantified by denaturing polyacrylamide gel electrophoresis on a 17% polyacrylamide, 5.8 M urea, 4% cross-linked gel. Gels were stained with ethidium bromide and visualized by UV illumination and densitometry (Printgraph AE-6932GXF, ATTO).

**DNA Cleavage Detection by MALDI-TOF Mass Spectrometry** DNA cleavage experiments were performed on a 100 μL scale with 2 μM PNA, 2 μM DNA oligonucleotide D3 (5'-AGTGATCTA-C-3'), 4 μM CuSO₄, 3 mM MPA (3-mercaptopropionic acid) in 10 mM phosphate buffer at pH 7.4. Reactions were incubated at 4°C for 48 h and quenched by addition of 10 μL of 20 mM EDTA. The DNA cleavage reaction mixtures were desalted using the ZipTip procedure. The oligonucleotides were bound to ZipTip, washed with 10 mM triethylammonium acetate buffer and eluted with 10 mM triethylammonium acetate–acetonitrile (1:1). The MALDI-TOF mass spectra were measured on an AB Sciex TOF/TOF™5800 system (AB Sciex) using 2,4,6-trihydroxyacetophenone containing diaminonium citrate (THAP/DAC) as the matrix.

**Molecular Modeling** Molecular modeling was performed by MacroModel (Schrödinger). X-Ray structure (PDB code: 1N8R) in ref. 23 was used as an initial structure. Models were built by replacing beta-hydrogen with the side chain possessing bipyridine unit, and conformation of the side chain was optimized with conformational search method of MacroModel.

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**Conflict of Interest** The authors declare no conflict of interest.

**References**