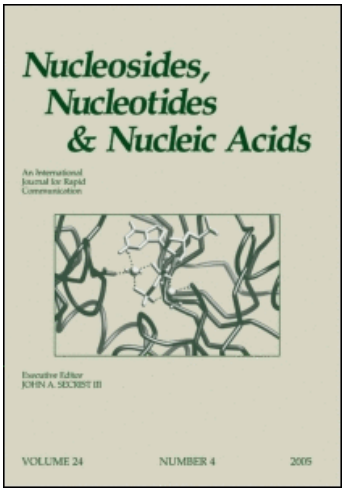

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
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Online publication date: 31 March 2001

To cite this Article Lonkar, P. S. , Kumar, V. A. and Ganesh, K. N.(2001) 'CONSTRAINED FLEXIBILITY IN PNA: DNA BINDING STUDIES WITH BRIDGED AMINOPROPYLGLYCYL PNA', Nucleosides, Nucleotides and Nucleic Acids, 20: 4, 1197 — 1200
To link to this Article: DOI: 10.1081/NCN-100002518
URL: http://dx.doi.org/10.1081/NCN-100002518

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS, 20(4–7), 1197–1200 (2001)

CONSTRAINED FLEXIBILITY IN PNA: DNA BINDING STUDIES WITH BRIDGED AMINOPROPYLGLYCYL PNA

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ABSTRACT

Introduction of methylene bridges in *aeg*PNA and *apg*PNA molecules give rise to cyclic five and six membered ring structures. Synthesis of a new six membered cyclic PNA monomer, aminopipecolyl PNA (*pip*PNA) is reported. Incorporation of *pip*PNA into PNA oligomers and comparative binding with target DNA sequences is studied.

Peptide nucleic acids (PNAs) are DNA mimics in which the deoxyribose phosphate backbone is replaced by a polyamide skeleton composed of *N*-(2-aminoethyl)glycine (*aeg*) units, with the nucleobases linked to the glycine nitrogen *via* an acetyl group (1). Because of their superior DNA-binding properties, PNAs have been used in many biological applications which require selective recognition of nucleic acid sequences (2). In order to improve the binding specificity, aqueous solubility and cellular uptake, the PNA backbone has been modified in many ways. One particular approach involves the introduction of chirality in PNA to discriminate the parallel/antiparallel orientation selectivity of binding to the target sequences (3).

Our earlier attempts in this direction involved introduction of a methylene bridge between the α carbon atom of the glycine moiety and the β carbon atom of the ethylenediamine segment to generate a five membered pyrrolidine ring (4) [aminoprolyl (*pr*) PNA] thus imparting structural rigidity to the PNA backbone

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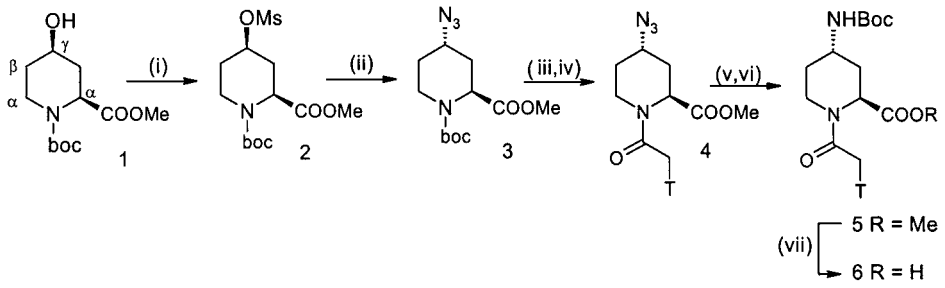
with simultaneous introduction of chirality. It was found that although the introduction of a single *pr*PNA unit at the N-terminus did induce directional selectivity of binding, the homooligomers comprising this monomer did not bind to the target sequences (5). The five membered rings in the *pr*PNA homooligomer seem to exert a conformational constraint and reduce the internucleobase distances. The effective *pr*PNA conformation is probably incompetent for binding to the target DNA sequence.

We report here a new PNA analogue by introducing a methylene bridge in aminopropylglycyl PNA (6), which has an aminopropyl instead of an aminoethyl segment in the backbone. Aminopropylglycyl PNA(*apg*PNA) is an extended flexible PNA analogue and binds to the target DNA sequences with much less efficiency compared to the *aeg*PNA (6). The proposed six membered monomeric unit formed as a result of the introduction of a methylene bridge between the γ carbon atom of the aminopropyl segment and the α carbon atom of the glycyl segment, *viz*. aminopipecolyl PNA(*pip*PNA) is expected to strike a balance between the flexibility and rigidity of the *apg*PNA backbone. The modification is aimed to relieve the constraint caused by the five membered ring in *pr*PNA, yet retaining the selectivity due to chirality. The synthesis of one (*trans* 2*S*, 4*S*) of the four possible diastereomers, its site-specific incorporation into PNA oligomers by solid phase synthesis and hybridization properties with complementary DNA is reported.

The chiral *pip*PNA thymine monomer *trans* 4*S*-(*N*-Boc-amino)-*N*-(thymin-1-ylacetyl)-2*S*-pipecolic acid **6** was synthesized from *N*-Boc *cis* 4*R*-hydroxy-2*S*-pipecolic acid methyl ester (7) as shown in Scheme 1. The 4*R*-hydroxyl group was converted to the 4*S*-azide derivative **3** *via* a mesyl intermediate **2** by S_{N^2} inversion of the C4 stereocentre. The ring nitrogen was deprotected and coupled with thymine acetic acid in presence of HOBt and DIPCI to yield compound **4**. The fully protected *pip*PNA monomer **5** was obtained after hydrogenation of the azide using Pd-C, followed by Boc protection of the resulting amino function. Hydrolysis of the ester **5** (8) afforded the monomer **6**. All the new compounds were characterized by ^{1}H NMR, ^{13}C NMR spectroscopic analyses and mass spectrometry.



(i) Py, MsCl (ii) NaN_3, DMF (iii) TFA, CH_2Cl_2 (iv) T-CH_2COOH, DIPCI, HOBt, DMF (v) H_2, Pd/ C (vi) Boc-N_3 (vii) 2N NaOH

Scheme 1.

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Table 1.

	PNA	UV-Tm°C X = prPNA	UV-Tm°C X = pipPNA
7	H-T_9**X**-β–ala-OH	64.8(b)	55.9(c)
8	H-T_3**X**T_5X-β-ala-OH	35.4	34.0
9	H-T_3**X**T_6-β-ala-OH	54.6	46.8
10	H-T_{10}-β-ala-OH	67.8(a)	
	DNA	5′-GC(A_{10})CG-3′	

Buffer: 10 mM sodium phosphate, pH 7.4. All Tms are correct to ±0.5°.

PNA oligomers **7–10** containing *aeg*PNA, *pr*PNA and the designed monomer *pip*PNA **6** at predetermined positions were assembled by solid phase peptide synthesis on Merrifield resin derivatized with *N*-Boc-β–alanine (0.26 meq/g of resin). The unmodified *aeg*PNA (denoted as T) sequence **10** is the control sequence. The other sequences **7–9** were designed so that the modified cyclic five membered *pr*PNA or six membered *pip*PNA monomers were incorporated either at the C terminal, internal position, or at both positions. The oligomers were cleaved from the solid support and purified using standard protocols (9).

The stability of the DNA:PNA_2 complexes was examined employing UV-melting studies (Table 1). A single *pr*PNA unit, when present at the C terminus is tolerated in the complex (ΔTm = 2°C, hyperchromicity 14%) much better than an *pip*PNA unit (ΔTm = 11°C, hyperchromicity 8%) (Fig. 1). The destabilization effect is more pronounced when a single modification is present in the middle of the sequence (ΔTm = 13°C & 21°C with less than 5% hyperchromicity for both *pr*PNA and *pip*PNA respectively). A very large destabilization was observed when two modified units were present in the PNA sequences (ΔTm ~ 32°C). Surprisingly the hyperchromicity change was high even when two five membered units were present in the PNA sequence although the overall complex stability was low. In

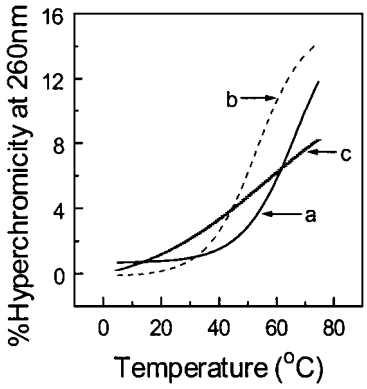

Figure 1.

general, the six membered ring system in the *api*PNA unit caused much larger destabilization of the DNA:PNA_2 complexes as compared to the five membered ring system. The preorganised conformations of the six membered ring structures in the present study, *trans* (2S 4S), seem to be incapable of adopting a structural bias required for efficient binding to DNA.

In summary, the new six membered *pip*PNA backbone bearing the nucleobase thymine has been synthesized and incorporated into PNA sequences. The modification was found to significantly impair the complexation of PNA with the complementary DNA sequence.

ACKNOWLEDGMENTS

We thank Prof. Yves Bousquet, Boehringer Ingelheim (Canada) Ltd. for providing **1** as a kind gift. PSL thanks Lady Tata Memorial Trust for a fellowship. VAK and KNG acknowledge Department of Biotechnology, New Delhi for a research grant.

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8. ^{1}H NMR ($CDCl_3$) δ: 7.3 (s, 1H), 7.0 (s, 1H), 5.4 (m, 1H), 5.0 (m, 1H), 4.7 (m, 1H), 4.3 (m, 1H), 3.8 (s, 3H), 3.5 (m, 2H), 2.6 (m, 1H), 2.1 (m, 1H), 2.0 (s, 3H), 1.5 (s, 9H). $[M]_{calc.} = 410$, $[M]_{obs.} = 411$.
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