Pure Appl. Chem., Vol. 76, Nos. 7–8, pp. 1599–1603, 2004.

Conformationally restricted cationic polyamide analogs of nucleic acids: Design, synthesis, and DNA/RNA binding studies*

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Abstract: The remarkable medicinal importance of the achiral, acyclic, and uncharged aminoethylglycyl peptide nucleic acids (*aeg*PNAs) as DNA/RNA mimics has challenged chemists to circumvent the limitations of their in vivo efficacy. In this context, we have designed conformationally restricted five- and six-membered cyclic PNA analogs by introduction of chemical bridges in *aeg*PNAs leading to a large variety of structures with defined configurations and conformational preferences, effecting concomitant installation of a positive charge in the backbone. The synthesis and biophysical properties of these cationic *aminoethylprolyl* PNAs, *pyrrolidine* PNAs, and *piperidine* PNAs endowed with increased water solubility and affinity toward target nucleic acids is presented. These nucleic acid analogs as lead structures are a part of a chemical evolution process that might give rise to a synthetic nucleic acid analog having optimum properties for medicinal applications.

INTRODUCTION

Chemical modifications of oligonucleotides have become synonymous with potential antisense therapeutics for the sequence-specific recognition and silencing of disease-causing genes [1]. These modifications are aimed at increasing the potency of the antisense/antigene oligonucleotides via increased nuclease resistance and cellular uptake [2]. The most important outcome of the search for chemically modified DNA/RNA **I** mimics is the emergence of specially designed achiral, acyclic, and uncharged aminoethylglycyl peptide nucleic acids (*aeg*PNAs) **II** (Fig. 1) based on molecular modeling studies [3].

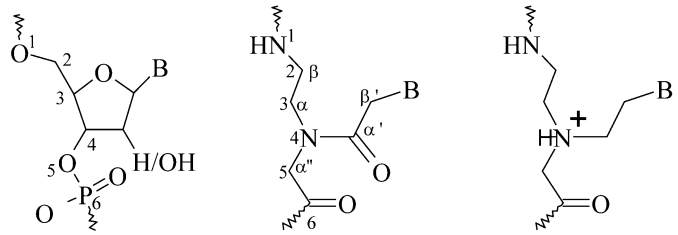

Fig. 1 Sugar-phosphate and aminoethylglycyl nucleic acids.

*Lecture presented at the symposium "Chemistry of nucleic acids", as part of the 39th IUPAC Congress and 86th Conference of the Canadian Society for Chemistry: Chemistry at the Interfaces, Ottawa, Canada, 10–15 August 2003. Other Congress presentations are published in this issue, pp. 1295–1603.

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The *aeg*PNAs recognize DNA/RNA with high sequence specificity following Watson–Crick (WC) and Hoogsteen (HG) hydrogen-bonding schemes and also exhibit unprecedented properties such as strand-invasion of duplex DNA. PNAs are also stable to nuclease and protease enzymes as the backbone carrying nucleobases is pseudopeptidic in nature. The application of these DNA mimics for therapeutic applications is limited by their poor water solubility, recognition of DNA/RNA sequences in both parallel or antiparallel orientation and inadequate cellular uptake. Various attempts have been made to address these shortcomings of PNAs [4]. Introduction of various modifications/substitutions in the PNA backbone in aminoethyl, glycyl, or acetyl segment carrying nucleobase leads to chiral PNA and is aimed at achieving directionally selective binding with target DNA/RNA. PNA appended with chiral, positively charged peptides and/or negatively charged nucleic acids is aimed at improving water solubility and cellular uptake of PNAs [5].

To circumvent the drawbacks of *aeg*PNA, our initial work [6] and that of others [7] led to the synthesis of conformationally restricted bridged analogs of *aeg*PNA-**II** that were cyclic, uncharged, and chiral structures, and were expected to bind to nucleic acids with parallel/antiparallel directional selectivity. However, these modified PNAs exhibited reduced binding affinity with complementary DNA. The excessive constraint could be the reason that these oligomers were not allowed to attain to a conformation congenial for binding to DNA. As a strategy to improve water solubility, *aeg*PNA-**III** was synthesized that was positively charged at physiological pH due to the presence of a tertiary amine group instead of a rigid uncharged tertiary amide nucleobase linker as in *aeg*PNA-**II** [8]. This caused *aeg*PNA-**III** to be highly soluble in water, but reduced binding affinity with target nucleic acids due to unrestricted conformational freedom. We then thought of combining together the two separate features in constructing bridged analogs of PNA-**III**, that led to the design of five- and six-membered cyclic, chiral PNA analogs with defined configurations and conformational preferences with concomitant installation of a positive charge in the backbone. In this strategy, the tertiary amide nucleobase linker is being replaced by a ring structure and thus the total elements of unsaturation are conserved as in *aeg*PNA-**II**. The information on the preferred torsion angles in the case of PNA:DNA complexes is limited [9] and therefore it is necessary to design a number of bridged structures and evaluate these for their DNA/RNA recognition properties. The position of the bridge that joins the aminoethyl, glycyl, or the nucleobase linkers (Fig. 2) and the stereochemistries of the resulting chiral heterocycles will dictate the conformations of the single-stranded (*ss*) PNAs. The constrained flexibility of the five- and rigid six-membered nitrogenous heterocyclic rings may be another factor in fine-tuning the *ss* PNA conformation. The desired conformation should be conducive for maximum enthalpic benefits from nucleobase recognition.

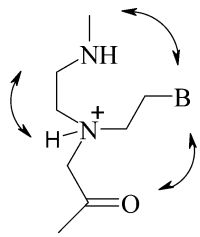

Fig. 2 Possible positions for the introduction of alkylene bridges.

Application of 4-hydroxy-L-proline for the synthesis of various PNA analogs

Naturally occurring 4-hydroxy-L-proline is a suitable starting material for the construction of cyclic PNA derivatives. It is readily available in optically pure form, and the five-membered ring is suitably functionalized at 2,4 positions that can be easily transformed in stereospecific manner to the required structural features leading to bridged PNA analogs. The single natural enantiomer also has a unique feature that allows its transformation to all four diastereoisomers arising due to the presence of two chiral centers. These stereochemical manipulations can be carried out using easy organic transformations like

lactonization and hydrolysis for C2 center and epimerization under Mitsunobu conditions of C4 center. The 4-hydroxy-prolinol also gives rise to a six-membered piperidine derivative by a stereospecific ring expansion reaction. We have utilized this unique functionalized cyclic amino acid for the construction of our designed PNA analogs.

*Aminoethylprolyl PNA (*aep*PNA)*

In the designed aminoethylprolyl PNA (*aep*PNA), the α″-carbon atom of glycine unit and the β′-carbon atom of the nucleobase linker were joined with a methylene bridge (Fig. 3). The nucleobase attachment to the pyrrolidine ring was fixed by virtue of the chirality of C4, unlike the possibility of rotameric populations in *aeg*PNA [10]. The monomers were synthesized in four steps starting from 4-*R*-hydroxy-2-*R*/*S*-proline. The pK_a of the tertiary amine function was found to be (~6.8) and the backbone could be at least partially protonated at physiological pH. The nucleobase having 4*S* stereochemistry was expected to have a similar spatial disposition as for the natural DNA. The oligomers comprising 4*S*,2*S/R aep*PNA thymine units showed very favorable binding properties toward the target sequences without compromising the specificity. The stereochemistry at C2 center did not exert any significant effect on the binding ability of the homo-oligomeric sequences. The mixed pyrimidine hairpin sequences with cytosine and *N*-7 guanine *aep*PNA units, exhibited directional discrimination in binding to parallel/antiparallel DNA sequences [10b,c]. The *aep*PNA units carrying individual nucleobases adenine, thymine, cytosine, and guanine in a mixed purine/pyrimidine sequence exerted nucleobase-dependent binding efficacies and orientation selectivities toward target oligomers [10d]. The results obtained so far using *aep*PNA are promising for their further biological applications. The binding specificity of these *aep*PNA oligomers in the sequence context needs further investigation and toward this aim, synthesis of stereoregular mixed purine/pyrimidine *aep*PNA sequences and their binding properties are currently underway in our laboratory.

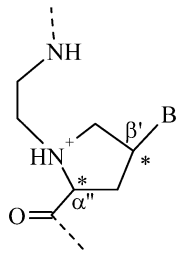

Fig. 3 Aminoethylprolyl PNA (*aep*PNA).

Pyrrolidine PNA I and II

Synthesis of pyrrolidine PNA-I was accomplished in our laboratory using the logic of exerting constrained flexibility in the *aeg*PNA-III through cyclic structures [11]. Insertion of a methylene bridge that linked α-carbon of the aminoethyl segment and β′-carbon of the tertiary amine nucleobase linker, led to the envisaged pyrrolidine PNA (Fig. 4). The monomer with 2*S*,4*S* stereochemistry was synthesized starting from *trans*-4-hydroxy-L-proline. The incorporation of the modified monomer into homothymine oligomers and mixed pyrimidine oligomers led to a decreased binding efficiency of these oligomers with the target DNA/RNA sequences. The 2*R*,4*R* isomer was synthesized and incorporated into a PNA:DNA dimer amenable for the synthesis of PNA:DNA chimera, by phosphoramidite chemistry. The chimeric PNA:DNA bound to the target DNA with decreased efficiency compared to the native DNA. The binding of chimeric PNA:DNA to target DNA could be improved by a five-atom linker between the modified units [11b].

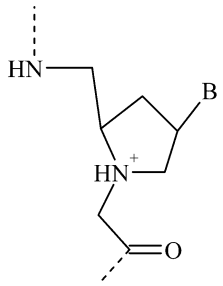

Fig. 4 Pyrrolidine PNA-I.

We further explored the possibility of introduction of β-α′-methylene bridge leading to another pyrrolidine-based PNA modification (Fig. 5) [12]. This may ease the rigidity of the direct attachment of the nucleobase to the pyrrolidine ring as in the case of the α-β′-methylene-bridged pyrrolidine PNA-I or *aep*PNA. Two thymine monomeric units with either 2*S*,4*S* or 2*R*,4*S* stereochemistry were synthesized from 4-*R*-hydroxy-2*S*/*R*-proline. It was observed that the 2*R*,4*S* pyrrolidine PNA monomer when incorporated in the center of the *aeg*PNA T_8 sequence was able to bind to the target DNA better than pure *aeg*PNA. Incorporation of other isomer with 2*S*,4*S* stereochemistry destabilized the complex with DNA. To our knowledge, this is the first report where such stereochemical discrimination was observed with a modified PNA. The tertiary amine function in this monomer being protonated at physiological pH may also affect the water solubility of the homo-oligomeric sequences. The results are preliminary, and further evaluation of this pyrrolidine PNA in the context of other mixed sequences is currently underway in our laboratory.

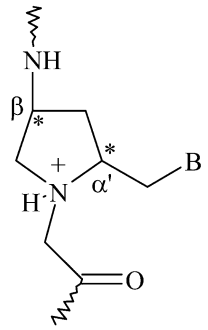

Fig. 5 Pyrrolidine PNA-II.

Piperidinyl PNA

We have now explored the possibility of introduction of β-β′-methylene bridge leading to a novel piperidine-based PNA modification (Fig. 6) [13]. The *trans*-4-hydroxy-L-prolinol gives rise to a six-membered piperidine derivative by a stereospecific ring expansion reaction. The resulting 3,5-dihydroxy derivative can be converted into protected PNA monomer that can be used for oligomer synthesis. This adds as a new repertoire to the designed PNA structures as the six-membered piperidine rings can be in a frozen chair conformation similar to hexitol nucleic acids and would therefore be quite specific in their binding preferences to target DNA. Our findings have indicated that the 3*S*,5*S*-piperidinyl PNA unit at the *C*-terminus stabilized the complexes with target DNA, whereas the same unit at the *N*-terminus was detrimental to this effect. Further work is currently underway.

In summary, the design and synthesis of the PNA structures with a variety of conformational and configurational possibilities is accomplished. The PNA analogs are positively charged at physiological

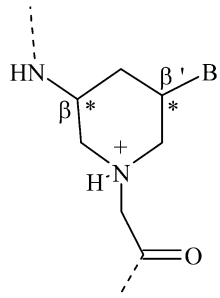

Fig. 6 Piperidinyl PNA.

pH because of the presence of tertiary amine group. This work presents a part of a chemical evolution process toward designed PNAs with optimum requisite properties.

ACKNOWLEDGMENTS

VAK thanks the Department of Science and Technology, Indian National Science Academy and National Chemical Laboratory for research and travel grants.

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