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COMMUNICATION

Synthesis of Amino-Acid-Derived Nucleo(side/tide) Analogs for Peptide-Derived Enantiospecific Nucleic Acid Analogs[1]

A convergent and cost-effective strategy to synthesize enantiospecific nucleoside/ nucleotide analogs from readily available α-amino acids has been described. Both (L)- and (D)-methionine were transformed in four steps to the corresponding key intermediates (L)- and (D)- 2-(tert-butyloxycarbonylamino)-4-bromomethyl butyrate. Nucleophilic displacement of the bromide by nucleic acid bases (e.g., adenine, thymine, guanine, cytosine) provided the enantiomerically pure monomers required for the solid-phase synthesis of novel peptide-derived enantiospecific nucleic acid analogs.

Therapeutic intervention against genetic expression that causes a pathogenic state, either at the transcriptional stage (antigene strategy) or at the translational stage (antisense strategy) by sequence-specific short synthetic oligo(deoxy)nucleotides (ODN/ONT), has remained an attractive and viable research strategy in the development of human therapeutics and diagnostic aids (*1*). Very promising preliminary results using antisense/antigene oligo(deoxy)nucleotides in cell culture have been reported, suggesting the potential of this methodology in inhibiting gene expression (*2, 3*). However, the development of therapeutically useful drugs from this line of research has proved to be a challenging task, mainly due to: (i) their inherent susceptibility toward cellular nucleases and, (ii) their poor transport across the cell membrane due to the intrinsic anionic nature of the sugar–phosphate backbone. Although there have been serious efforts made to modify the natural phosphodiester internucleotide linkage [as reviewed in (*1, 4*)], these efforts have so far met with only limited success.

Another approach to these problems could be to replace the entire sugar–phosphate backbone by an amide or peptide backbone. Such a modification would involve assembling of base-containing monomeric subunits via amide/peptide linkages (Fig. 1). In addition to their expected advantage of being resistant to cellular nucleases, their electrostatically neutral backbone should tend to increase uptake through cellular membranes. Such amide/peptide backbone-derived oligodeoxynucleotide analogs also lend themselves to preparation in large quantities by solid phase synthesis.

The concept of peptide backbone-derived nucleic acid analogs has been in the

[1] An abstract of this work was submitted to the Division of Medicinal Chemistry on December 9, 1994 and was later presented in part as a poster (MEDI 228) at the 209th American Chemical Society National Meeting held in Anaheim, CA, April 2–6, 1995. A U.S. patent for this work also has been applied for and is pending approval.

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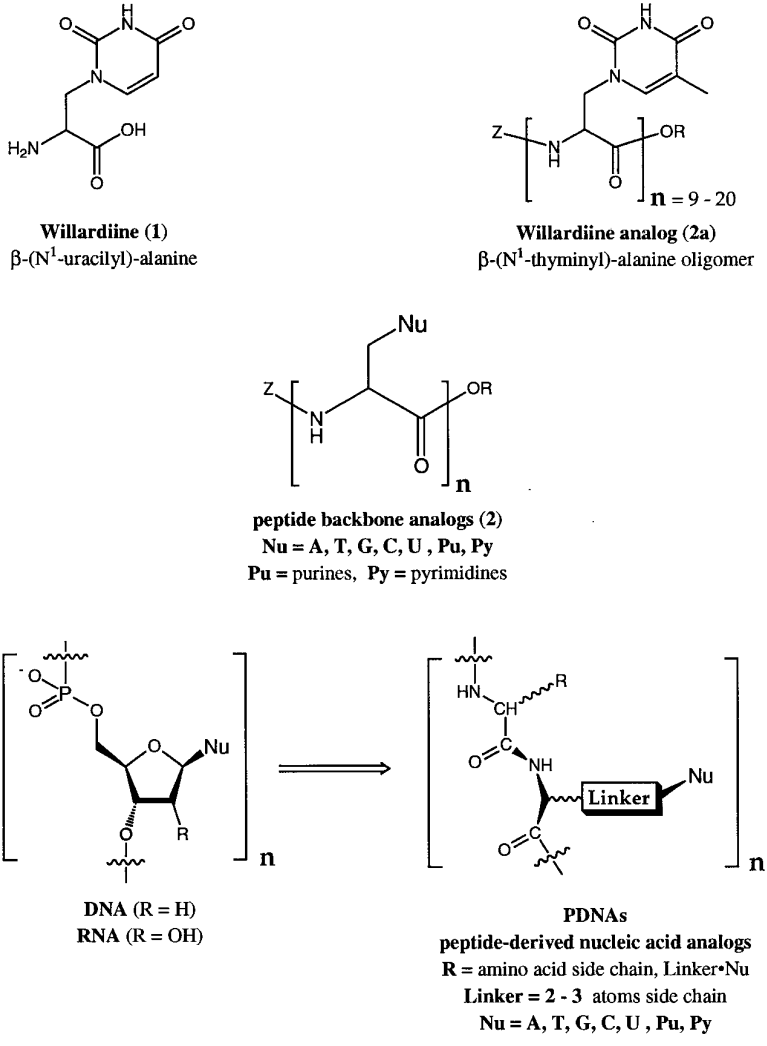

FIG. 1. Peptide backbone analogs and DNA/RNA vs PDNAs.

literature since the early 1970s. Their development was motivated by the unique structure of Willardiine [β-(N1-uracilyl)alanine, **1**], a natural product isolated from the seeds of *Acacia Willardiana* Rose (*5*). Willardiine contains one of the recognition elements of a nucleic acid, namely uracil as a side chain substituent on the beta carbon of alanine. It was apparent that, in principle, an oligomer with a proper set of the nucleic acid base analogs of **2** (Nu = A, T, G, C, U, Pu, Py) could offer the potential of recognizing complementary sequences on the target nucleic acid. Doel *et al.* and Buttery *et al.* (*6, 7*) were the first to conceptualize this notion and synthesize oligomers in which the uracil was replaced with thymine (**2a**). However, they failed

to demonstrate effective binding of **2a** to its complementary ODN, polyadenylic acid (Poly-A). Since then there have been numerous efforts made by several other groups to synthesize analogs with amide/peptide backbones (*8–15*). Some of these analogs have met with limited success and others have the potential to become human therapeutic agents (*12, 14, 16, 17*).

Except for a handful of examples (*12, 14, 17*), most of the amide/peptide backbone-derived nucleic acid analogs reported so far in the literature are either racemic or quasichiral [e.g., side chain is attached to nitrogen rather than the α-carbon (*16*)]. Both natural DNA and RNA are enantiomerically pure molecules, and, from our preliminary molecular modeling studies, we felt that an enantiospecific assembly of monomers might offer some advantages in maintaining Watson–Crick/Hoogsteen base-pairing to the complementary nucleic acid over the racemic or achiral analogs. Recognizing these potential differences, we set out to redesign and evaluate this general approach.

In nucleic acids, the purine and pyrimidine bases are oriented away from the sugar–phosphate backbone toward the helical axis and are stacked with a periodicity approximately 3.5 Å from each other. It seems essential that to retain the sequence-specific recognition and efficient binding to its complementary ONTs or target nucleic acid, a peptide-derived nucleic acid analog should be structurally isomorphous to DNA/RNA, i.e., the distance between the purine and pyrimidine bases from the peptide backbone and also the distance of the bases from each other ideally should be similar to that in native DNA/RNA. In other words, the synthesis of such a oligopeptide would require substitution of purine and pyrimidine bases at least at three bonds distant (i.e., at the γ-carbon) from the α-carbon of an amino acid monomer. In addition, it seems that the adjacent nucleic acid bases in such a peptide-derived nucleic acid analog should ideally be oriented ''inward'' (i.e., toward the helical axis) in contrast to their preferred natural ''outward'' (i.e., away from the helical axis and the backbone) orientation by incorporation of both (L)- and (D)-enantiomers of amino acids as the monomer units instead of either racemic (DL) or the (L)- or (D)-enantiomers alone.

From these considerations, our redesigned peptide-derived nucleic acid analog emerges as an oligopeptide composed of optically pure (L)- and (D)-amino acids as monomers, in which the nucleic acid bases or analogs thereof are substituted at the γ-atom of the side chain. Since these molecules feature the elements of both nucleic acid (i.e., nucleic acid bases: A, T, G, C, U) and peptide (i.e., amide backbone) they could be considered as chimeras between nucleic acids and peptides. We would prefer to call them **p**eptide-**derived n**ucleic acid **a**nalogs (**PDNAs**) in general, since it describes both their origin and function. Although there have been a few examples in the literature incorporating either (*RS*)- or (*S*)-, or (*R*)-stereoisomers of monomers (*14, 17*), there is no reported precedent of such enantiospecific oligopeptides incorporating both (*S*)- and (*R*)-enantiomers in such a fashion in the molecule.

We decided to synthesize a model **PDNA,** a homo decapeptide (Scheme 1, **I–III**), in which the nucleic acid bases are appropriately appended to the α-carbon by an ethylene linker. A retrosynthetic analysis (Scheme 1) suggested that the synthesis of such PDNAs would require optically pure (L)- and (D)-homoalanines, substituted