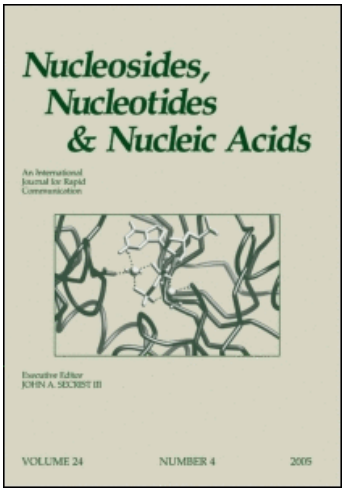

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A SIMPLE NUCLEIC ACID ALTERNATIVE: AMINOPROPYL NUCLEIC ACIDS (APNAs)

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□ *Aminopropyl nucleic acids are constitutionally simple nucleic acids alternatives with one chiral center per nucleotide and with the constitutional potential to hybridize with RNA. Both R and S isomers of the 3′- or 2′-aminopropyl nucleosides were incorporated either into DNA or likewise were used for fully modified sequences. The (R)-adenine analogue, yielding (S)-APNA, can be considered as a candidate for universal base pairing.*

Keywords Aminopropyl nucleosides; antisense oligonucleotides; nucleoside phosphoramidates

INTRODUCTION

For two decades already, antisense chemistry and the potential inhibitory effects of antisense oligonucleotides on gene expression have aroused large scientific interest. In addition, a systematic approach is undertaken to try to understand the chemical etiology of the present-day nucleic acids. Along these lines, A. Eschenmoser and his colleagues developed threose nucleic acids (TNA) and found that complementary TNA strands could form stable double helices. The TNA strands also pair up with complementary strands of RNA and DNA.[1,2] Our interest in potential antisense reagents prompted us therefore to design an "open-chain"-TNA analog, using aminopropyl nucleosides as building blocks. Such simplified analogs contain only one chiral center, and one could ask the question whether chirality would still play an important role in the strength and selectivity of hybridization. Therefore, in order to evaluate their chemical stability and hybridization properties, we decided to prepare both constitutional series (2′-amino- and 3′-aminopropyl nucleic acids) in both chiral forms (*R* or *S* monomers) with both the adenine base or the thymine base, resulting in 4 different series of oligonucleotides (Figure 1). The important new findings

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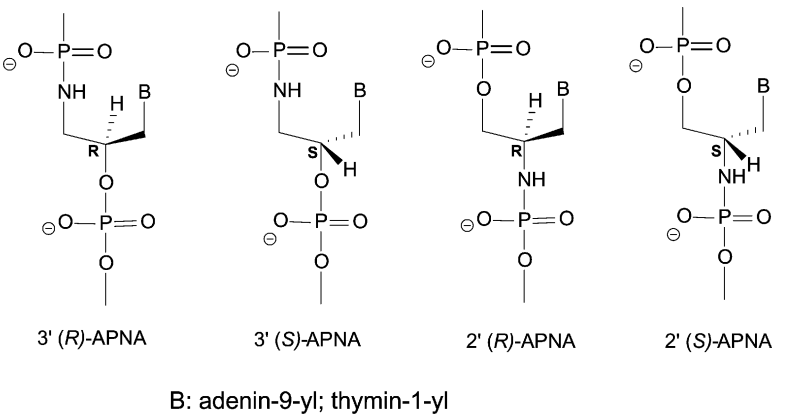

FIGURE 1 Overview of the possible acyclic phosphoramidate linkages.

are the use of the biphenylyl-propyloxycarbonyl protecting group (Bpoc), the 2′-aminated analogs, and the assembly of fully modified sequences for each series.

RESULTS AND DISCUSSION

The optically active 3′- or 2′-aminopropyl nucleosides (APN) were both prepared from enantiomerically pure (*S*)-1,2-*O*-isopropylidene-glycerol.[3,4] To circumvent the assembly problems, at first preformed dimers containing an acyclic phosphoramidate block were used. To reduce however the previously experienced nucleophilicity of the nitrogen atom during the assembly process,[3] we now introduced the Bpoc protecting group. Two classes of Bpoc protected and phosphitylated 3′-aminopropyl- and 2′-aminopropyl nucleoside building blocks (3′ and 2′-APNps) were synthesized and could be used for the preparation of the corresponding fully modified oligonucleotides containing exclusively phosphoramidate linkages.[5]

Their assembly was accomplished using a modified version of the phosphoramidite method. Improved yields for the amine-exchange reaction were obtained using a double coupling approach (couple-oxidize-couple-cap-oxidize). The detachment from the solid support with concomitant deprotection was achieved as usual by treatment with methylamine (40% in water) and concentrated aqueous ammonia (1:1, 30°C). Purification by ion exchange chromatography and analysis by mass spectrometry were cumbersome, but proved the correct assembly of the phosphoramidate building blocks.

Pairing Properties of the Acyclic Phosphoramidates

The incorporation of a single **T*** or **A*** modification (3′-aminated with either *R* or *S* chirality) within a DNA strand resulted already in a strong decrease of the stability (ΔT_m/mod = −9.8 to −15.7) as shown before (incorporation via preformed dimer units).[3] The loss in affinity was dependent on the sequence and proved larger for incorporation of a **T*** block than for **A*** substitutions. When analyzing **T*** and **A*** modifications opposing each other in complementary DNA strands within an A/T stretch (5′-CCT$_9$CC/GGA$_9$GG), very strong destabilization (−20°C) was noticed for the acyclic "pairs" **T*/A*, A*/T***, **T*/T*** but to a lesser extent for the more voluminous **A*/A***. Remarkably, we observed the hybridization properties of the (*S*)-APNA (incorporation of (*R*)-acyclic blocks)* to be slightly favoured, indicating that the chirality of the carbon atoms of APNA plays an important role in hybridization strength when incorporated within a dsDNA sequence.

To further evaluate the discrimination properties for **T*** and **A***, a single modification (**X**) was likewise incorporated into a mixed sequence context (5′-CACCG**X**TGCTACC/3′-GTGGC**Y**ACGATGG) and hybridized to the four natural bases (**Y**). The T_m spread for the different mismatches proved smallest for the **A*** congeners, with again best results obtained for the (*R*)-**A*** nucleoside analog ((*S*)-APNA series). This modified building block indeed displayed almost universal or ambiguous character in hybridising to all four natural bases with about equal strength, with a spread of only 2.7°C.

The new synthetic strategy with the Bpoc group, however, allowed incorporation of consecutive acyclic analogs within homothymidine or homodeoxyadenosine polymers [(dT)$_{13}$ or (dA)$_{13}$, respectively]. Unfortunately, incorporation of 3 to 5 consecutive **T*** modifications proved detrimental for the pairing potential, and no interaction with either a DNA or RNA complement could be detected anymore. As multiple changes in the number of connecting atoms between consecutive bases could be responsible for the detrimental pairing, we decided to study only fully modified sequences anymore [(**T***)$_{13}$ or (**A***)$_{13}$] for the different series [3′(*R*)- and 3′(*S*)-APNA and 2′(*R*)- and 2′(*S*)-APNA]. Synthesis proved cumbersome and yielded complicated HPLC profiles with only low isolated yields for most final products. The small quantities available of the fully modified sequences were used to study self-pairing (**A*/T***, **T*/T*** **A*/A***), cross-pairing between the different series (like 2′(*R*)-**A*** with 2′(*S*)-**T*** APNA), as well as interaction with DNA and RNA complements. Unfortunately, no interactions could be detected by thermal denaturation experiments.

*Introduction of the 3′-amine changes the priority rules, but attachment of a phosphate at the nitrogen atom affords (*S*)-APNA from (*R*)-acyclic blocks.

CONCLUSION

Incorporation of consecutive acyclic phosphoramidate building blocks in homothymidine polymers showed a strong decrease in stability of the corresponding dsDNA. Upon evaluation of fully modified sequences, unfortunately no interactions could be detected by thermal denaturation experiments neither for self-pairing, homochiral pairing between APNAs or cross-pairing with DNA or RNA.

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