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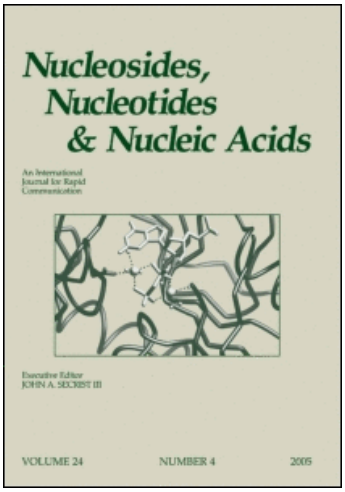

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*dab*Pna: Design, Synthesis, And Dna Binding Studies

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*dab*PNA: DESIGN, SYNTHESIS, AND DNA BINDING STUDIES

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□ *In continuing our research efforts for developing new oligodeoxynucleotide (ODN)-like drugs and diagnostics, we designed diaminobutyric peptide nucleic acids (*dab*PNAs), nucleopeptides characterized by a diaminobutyric-based building block that is an isomer of the aminoethylglycyl PNA (*aeg*PNA) unit and the acyclic modification of the aminoprolyl PNA (*amp*PNA) monomer. In this work we present the solid phase synthesis of a* dab*PNA oligomer and of two* aeg*PNAs containing a single* dab*PNA unit. A study relative to their binding ability towards DNA is also reported even in comparison with the well known* aeg*PNAs.*

Keywords DABA; nucleopeptides; binding studies

INTRODUCTION

The improvement in *aeg*PNA (**1**, Figure 1)[1] characteristics, like water solubility, cellular uptake and discrimination between parallel and antiparallel binding modes, is clearly desirable and in some cases can be achieved by various modifications in the *aeg*PNA submonomeric unit. The introduction of chirality and also of positive/negative charges in the PNA backbone sometimes improved PNA properties.[2] In particular, *aeg*PNAs with a single aminoprolyl PNA unit (**3**, Figure 1) at N-terminus showed discrimination of antiparallel versus parallel binding to DNA and an alternate *aeg/amp*PNA showed higher binding affinity than pure *aeg*PNAs.[3]

In continuing our research for the development of new ODN-like drugs or diagnostics, we designed and realized a chiral *dab*PNA (**2**, Figure 1), a nucleo-γ-peptide characterized by a 2,4-diaminobutyric acid (DABA)-based

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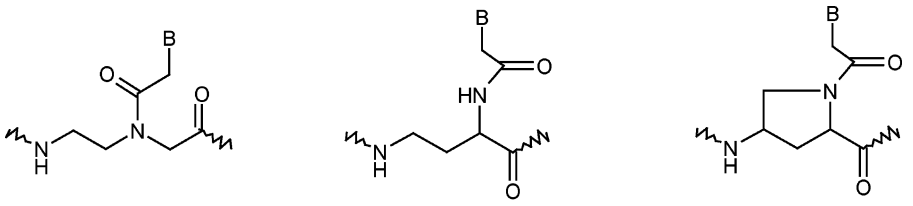

*aeg*PNA 1 *dab*PNA 2 *amp*PNA 3

FIGURE 1 *dab*PNAs are characterized by a chiral γ-peptide skeleton instead of the pseudopeptide backbone of *aeg*PNAs and are more flexible than their cyclic analogues *amp*PNAs.

building block that is an isomer of the *aeg*PNA unit (**1**) and the acyclic modification of the *amp*PNA monomer (**3**). Our interest to the presented *dab*PNA was supported by the known stability of γ-peptides to enzymatic degradation and also by the proposal formulated by Meierhenrich et al.[4] of DABA-based PNAs as prebiotic material in a primordial "PNA world" that would have preceded our present-day "two-polymer world." This hypothesis followed the recovery of DABA in the extraterrestrial soil of the Murchison meteorite together with other diaminoacids,[4] in our opinion potential candidates for the realization of new PNAs.

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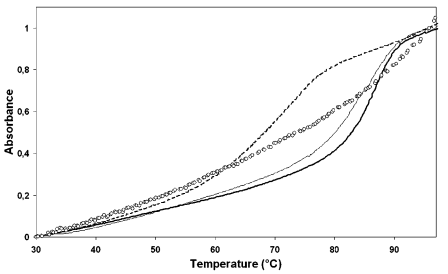
RESULTS AND DISCUSSION

We chose, as starting material for the synthesis of *dab*PNA nucleopeptides, the L-enantiomer of the diaminobutyric acid, in analogy to the approach of Petersen et al. in the case of the ornithine-based PNAs.[5] The thymine containing monomer was synthesised starting from the commercially available Boc-(*S*)-DAB(Fmoc)-OH diaminoacid. In the first synthetic step the Boc group was selectively removed with trifluoroacetic acid at 50°C to give the free amino group in α position (90% yield). The obtained product [ESI-MS *m/z*: 340.4 (found), 341.38 (expected for MH^+)] was coupled with the thymine-1-acetic acid under different synthetic conditions with the best results coming from the use of HATU/DIEA in DMF as solvent (72% yield).

The new Fmoc-protected monomer (t_d), characterized by LC-ESIMS [*m/z*: 507.32 (found), 507.52 (expected for MH^+)] and ^1H and ^{13}C-NMR spectroscopy, was oligomerized manually on solid phase to the corresponding *dab*PNA dodecamer (**4**, Figure 2b) following a protocol used by Sforza et al. that minimize racemization during solid phase synthesis of chiral PNA analogues.[6] The t_{12} *aeg*PNA (**5**) also was synthesized as reference oligomer for hybridization studies. Furthermore, two dodecamers carrying a single

a)



b)

Sequences	Tm (°C) ± 0.5
H-G-$(t_d)_{12}$-K-NH_2 (**4**)	—
H-G-$(t)_{12}$-K-NH_2 (**5**)	86.2
H-G-$t_d(t)_{12}$-K-NH_2 (**6**)	84.8
H-G-$(t)_6t_d(t)_5$-K-NH_2 (**7**)	72.0

FIGURE 2 a) UV melting curves for the duplexes formed between dA_{12} and the PNA oligomers: **4** (open circle), **5** (solid line), **6**, (thin line), and **7** (dashed line); b) T_m values relative to the adjacent melting profiles.

t-*dab*PNA unit in the *aeg*PNA chain were realized to study the influence on the ability of *aeg*PNAs to bind natural nucleic acids, when the new monomer is inserted in different chain positions (**6** and **7**). All the oligomers were purified by semipreparative RP-HPLC, quantified by UV and characterised by LC-ESIMS. Overall yields of all the PNA dodecamers were about 20%. ESI-MS *m/z* expected for oligomers **4–7**: 1133.4 ($[M+3H]^{3+}$), 1699.7 ($[M+2H]^{2+}$). ESI-MS *m/z* found for oligomers **4–7**: 1134.8 (**4**); 1132.0, 1697.1 (**5**); 1133.0, 1699.8 (**6**); 1132.8, 1698.5 (**7**).

The ability of **4–7** to bind a dA_{12} was studied by UV melting experiments (Figure 2).

From our preliminary results, the *dab*PNA homopolymer **4** seems not to bind the complementary dA_{12} (open circle, Figure 2a) and the incorporation of *dab*PNA monomer at N-terminus or in the middle of the homothymine *aeg*PNA oligomer leads to a decreased binding efficiency for the oligomers **6** and **7** to the target DNA sequence (T_m 84.8 and 72.0°C, Figure 2b) in comparison to *aeg*PNA **5** (T_m 86.2°C).

Furthermore, CD hybridization studies were carried out by Tandem mix cell on **4** with dA_{12}. Unlike oligomers **5**, **6**, and **7**, t_{12} *dab*PNA **4** didn't show any difference between the sum CD spectrum, obtained before mixing the two components, and the complex CD spectrum, recorded after the mixing, as one would expect in case of binding with dA_{12}.

Even if no encouraging results, relative to the ability of L-*dab*PNA to bind DNA came from the current investigation, D-*dab*PNA will be studied analogously to this work. Furthermore, since alternate *amp*PNA/*aeg*PNA strands showed very favourable DNA hybridization properties (better than *aeg*PNA), taking into account that *dab*PNA monomer is the acyclic counterpart of the *amp*PNA one, we intend to synthesize alternate *dab/aeg*PNA. Finally, modelling studies on polymers based on the other diaminoacids identified in Murchison meteorite[4] are ongoing in order to select the best candidate as new antigene/antisense tool.

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