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2'-Deoxyribo-PNAs: A Structurally Novel Class of Polyamide Nucleic Acids with Good RNA and DNA Binding Affinity

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Abstract: 2'-Deoxyribo polyamide nucleic acids (2'-deoxyribo-PNAs) **3** are a new class of DNA analogs with a 2',3'-dideoxyribose-polyamide backbone structure. 2'-Deoxyribo-PNAs as well as chimeric oligonucleotide analogs with a mixed DNA / 2'-deoxyribo-PNA structure bind to single stranded complementary nucleic acids with similar affinities as natural DNA.

The replacement of the natural phosphodiester linkage in oligonucleotides (**1**, *Figure 1*) with neutral, non-phosphorus containing linker moieties[1] has been pursued as a potential approach to antisense drugs.[2] In this context, certain amide units (*e.g.* of type **3**) have been described as replacements for *non-contiguous* phosphodiester groups.[3] The resulting oligodeoxynucleotide analogs have been reported to show moderately enhanced RNA-, but not DNA-binding affinity.[3] A more rigorous approach towards amide-modified nucleic acid analogs has led to the development of peptide nucleic acids (PNA, **2**), which are entirely based on a polyamide backbone composed of repeating aminoethyl glycyl units.[4]

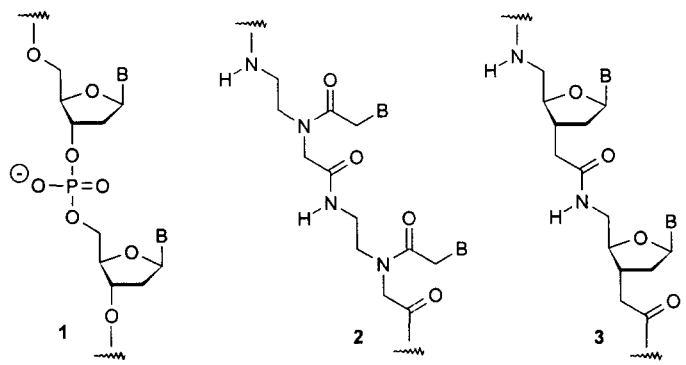

Figure 1.

Here, we describe a structurally novel type of polyamide nucleic acid that is based on a uniform 2'-deoxyribose polyamide backbone of type **3**. In addition, we have also investigated chimeric analogs in which only parts of the deoxyribose-phosphodiester backbone have been replaced by stretches of consecutive amide linkages.[5] The stability of duplexes formed by both types of oligodeoxynucleotide analogs was found to be similar to that of natural RNA/DNA or DNA/DNA hybrids.

Oligonucleotide analogs **I** - **IV** (see *Table 1*) were all synthesized by solid-phase methods employing N_δ-monomethoxytrityl (MMTr)-protected amino acid derivatives. As illustrated in *Scheme 1*, the requisite

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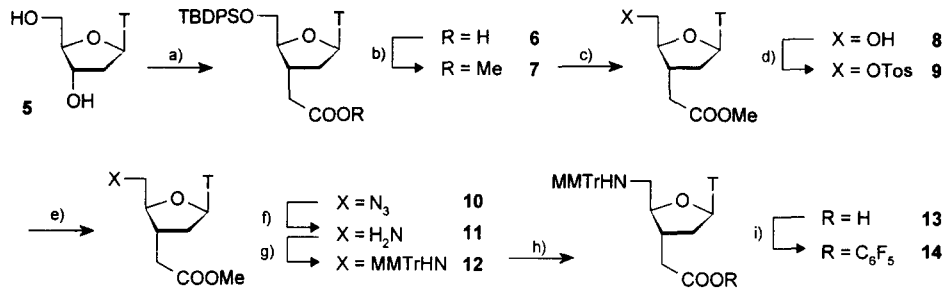
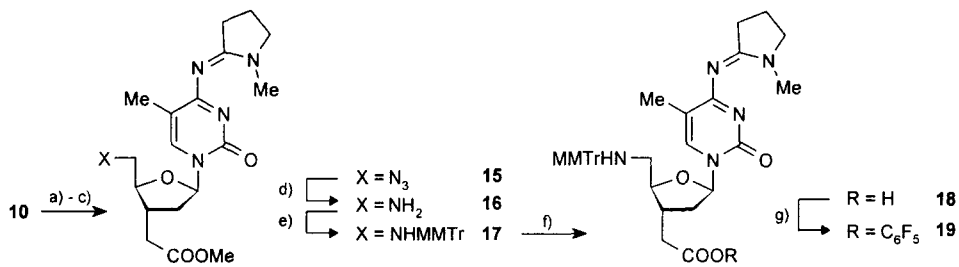
thymidine unit **14** was prepared from O(5') protected carboxylic acid **6**, which can be accessed from thymidine according to published procedures.[3] **6** was elaborated into tosylate **9** by DCC-mediated methyl ester formation, followed by tetrabutylammonium fluoride-induced O(5') desilylation and reaction of the resulting hydroxy ester **8** with tosyl chloride. Heating of a DMF solution of **9** to 100 °C in the presence of lithium azide[6] afforded azide **10**, which was reduced with H_2/Pd-C to the amine **11** and converted to **12** by treatment with MMTr-Cl. The methyl ester was then hydrolyzed to the acid **13** (LiOH, THF/H_2O), which was reacted with pentafluorophenyl trifluoroacetate[7] to yield the desired activated ester **14**.



Scheme 1. Synthesis of pentafluorophenylester **14**. a) Ref. 3. b) 1.1 eq DCC, 10 eq MeOH, 0.8 eq DMAP, CH_2Cl_2, rt, 16 h, 89%. c) 2.0 eq Bu_4NF, THF, 0 °C, 3 h, 78%. d) 4 eq Tos-Cl, 5 eq pyridine, cat. DMAP, $CHCl_3$, 40 °C, 16 h, 83%. e) 4.0 eq LiN_3, 2.0 eq NaI, DMF, 100°C, 16 h, 78%. f) 0.1 eq Pd/C (10%), H_2 (1 bar), MeOH, rt, 16 h. g) 1.2 eq MMTr-Cl, pyridine, rt, 16 h, 75% (2 steps). h) 10 eq LiOH x H_2O,THF / H_2O (4 : 1), RT, 3 h. i) 1.5 eq C_6F_5-OOCCF$_3$, 20 eq pyridine, DMF, rt, 6 h, 50% (2 steps).



Scheme 2. Synthesis of pentafluorophenylester **19**. a) 2.5 eq $POCl_3$, 22 eq NEt_3, 20 eq 1.2.4-triazole, MeCN, 0°C → RT, 2 h, quant. b) 50 eq NH_3 (25% aq), 0°C → rt, 1 h, 99%. c) 6 eq *N*-methylpyrrolidone dimethylacetal, 6 eq pyridine, MeOH, rt, 4 h, 75%. d) 0.1 eq Pd/C (10%), H_2 (1 bar), MeOH, rt, 3 h. e) 1.2 eq MMTr-Cl, pyridine, rt, 16 h, 43% (2 steps). f) 10 eq LiOH x H_2O, THF / H_2O (4 : 1), rt, 6 h. g) 1.5 eq C_6F_5-OOCCF$_3$, 20 eq pyridine, DMF, rt, 6 h, 31% (2 steps).

The synthesis of the activated 5-methyl cytidine unit **19** (*Scheme 2*) involved conversion of the azido ester **10** to the *N*(4)-(*N*-methylpyrrolidin-2-ylidene)-protected 5-methyl cytidine derivative **15**.[8, 9, 10] Reduction of the azide moiety to the amine **16** and subsequent MMTr protection gave methyl ester **17**, which was elaborated into the pentafluorophenylester **19** in analogy to the preparation of **14**.

The solid-phase synthesis of oligomers **I** - **IV** was carried out on controlled-pore glass (CPG) using a standard succinate linker or a β-eliminative support (for **I**), respectively.[11] Amide bond formation was achieved by reacting the free 5'-amine functionality, generated from the MMTr-protected compound by brief (1 min) treatment with 3% dichloroacetic acid in methylene chloride, with the pentafluorophenylesters **14** or **19** in the presence of an excess of *N*-hydroxybenzotriazole[12] and *sym*-collidine. Coupling times of 30 minutes resulted in an average coupling yield of 98 - 99% per step, as determined by UV spectrophotometric determination of the concentration of the released MMTr cation. The phosphodiester-based regions of the DNA - 2'-deoxyribo-PNA hybrids **II** - **IV** were prepared using standard phosphoramidite chemistry,[11] but with prolonged coupling times of 10 minutes. Building blocks **20** - **23**[13] were used to establish the transitions between DNA and 2'-deoxyribo-PNA stretches. Cleavage of the oligonucleotide analogs from the solid

support with concomitant base deprotection was achieved by overnight treatment with 25% aqueous ammonia at room temperature. In order to increase the water solubility of the neutral 2'-deoxyribo-PNA **I**, both the 3'- as well as the 5'-terminal hydroxyl group were derivatized as negatively charged phosphates.[14, 15]

Table 1: 2'-Deoxyribo-PNA modified oligonucleotide analogs investigated in this study.[a]

pTxTxTxTxTxCxTxCxTxCxTxCxTxCxTp	**I**
TxTxTxTxTxC T C T C T C T C T	**II**
T T T T T C T C T CxTxCxTxCxT	**III**
T T T T TxCxTxCxTxCxT C T C T	**IV**

[a] "p" in analog **I** indicates a terminal phosphate group. "C" signifies modified building blocks incorporating 5-methylcytosine as the base. "x" indicates an internucleoside amide linkage of the type 3'-CH_2-CO-NH-CH_2-4'.

The melting temperatures (T_m) of the duplexes formed by analogs **I** - **IV** with complementary DNA and RNA strands are summarized in *Table 2*. In all UV melting experiments, melting profiles indicative of a single cooperative transition were obtained without hysteresis upon cooling.[16] All four analogs bound to their RNA *and* DNA complements with affinities not significantly different from those observed for the respective unmodified wild-type oligodeoxyribonucleotides (*Table 2*). It was intriguing to find that the *relative* stability of the duplex of the fully modified 2'-deoxyribo-PNA **I** with DNA (ΔT_m = -3.0 °C) was higher than that of the corresponding **I** / RNA duplex (ΔT_m = -6.0 °C). In this context, it is important to recall that oligonucleotide analogs containing an alternating arrangement of amide and phosphodiester linkages generally bind to complementary RNA strands with higher affinity than the corresponding unmodified oligodeoxyribonucleotides, whereas their DNA-binding affinity is significantly diminished.[3c] Regarding base-pair specificity, the T_m of the duplex between **IV** and a complementary oligoribonucleotide containing a single mismatched base at position 9 (*r*(AGAGAGAG*C*GAAAAA)) was 38.3 °C, i.e. 11.4 °C lower than for the matched **IV** / RNA duplex (*cf. Table 2*). This compares well with a drop in T_m of 10.4 °C for the binding of the respective wild-type oligodeoxyribonucleotide to the same mismatched RNA (43.8 °C *vs.* 54.2 °C) and indicates that base-pair specificity is not compromised for a polyamide backbone of type **3**.

Table 2: Melting temperatures (T_m [°C]) of duplexes of modified oligonucleotides **I** - **IV** with their RNA and DNA complements. [a], [b], [c], [d]

Sequence	**I**	**II**	**III**	**IV**
T_m vs. RNA[e]	51.0	54.1	51.4	49.7
(ΔT_m / mod.)	(-0.4)	(+0.3)	(-0.7)	(-0.8)
T_m vs. DNA[f]	46.5	40.5	44.0	43.7
(ΔT_m / mod.)	(-0.2)	(-0.6)	(-0.5)	(-0.5)

[a] For structures of analogs **I** - **IV** *cf. Table 1*. [b] ΔT_m/mod: [T_m (oligonucleotide analog) - T_m (wild type oligonucleotide)] / (number of amide linkages). [c] The extinction *vs.* temperature profiles were measured for each strand (4 mM) in 10 mM phosphate (pH 7.0, Na^+ salts) with 100 mM total [Na^+] (supplemented as NaCl) and 0.1 mM EDTA. [d] Reference wild-type oligonucleotides (**I'** - **IV'**) are the fully PO-based oligodeoxyribonucleotides corresponding in base sequence to analogs **I** - **IV** and all contained 5-methyl dC and terminal phosphate groups, respectively, at the appropriate positions. [e] Wild-type T_m's are as follows: **I'**: 56.1; **II'**: 52.6; **III'**: 54.7; **IV'**: 54.2. [f] Wild-type T_m's are as follows: **I'**: 49.2; **II'**: 43.5; **III'**: 46.5; **IV'**: 46.9.

In summary, novel 2'-deoxyribo-PNAs have been efficiently synthesized on solid support using MMTr-protected monomeric building blocks **14** and **19**. The resulting oligodeoxyribonucleotide analogs, either as 2'-deoxyribo-PNA - DNA hybrids or as fully modified 2'-deoxyribo-PNAs, show good binding affinity towards *both* complementary RNA *and* DNA strands. Extension of the chemistry reported herein to all four nucleic acid bases and evaluation of additional biologically relevant sequences are under current investigation.

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10 Just *et. al.* have independently synthesized a *N*(4)-benzoyl protected cytidine derivative corresponding to **15**. However, they did not report further elaboration of this compound. See: Grunder-Klotz, E.; Just, G. *Nucleosides & Nucleotides* **1994**, *13*, 1829 - 1841.

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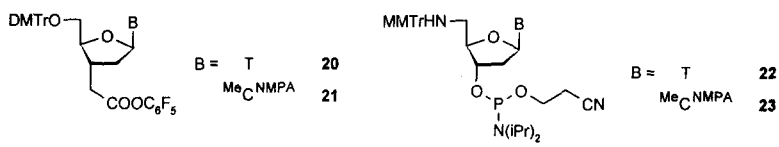
13 Activated ester **20** was also employed for the incorporation of the 5'-terminal thymidine unit in analog **I**, while a building block related to **22** was used for the 3'-terminal thymidine unit in **III**. For **22**; see: Bannwarth, W. *Helv. Chim. Acta* **1988**, *71*, 1517 - 1527.



For an automated solid-phase synthesis of PNA-(5')-DNA-(3')-PNA chimera, see also: Van der Laan, A. C.; Brill, R.; Kiumelis, R. G.; Kuyl-Yeheskiely, E.; Van Boom, J. H.; Andrus, A.; Vinayak, R. *Tetrahedron Lett.* **1997**, *38*, 2249 - 2252.

14 Phosphorylations were achieved according to previously published procedures and involved use of a β-eliminative support for the solid-phase synthesis of **I** (introduction of the 3'-phosphate group; Efimov, V. A.; Buryakova, A. A.; Reverdatto, S. V.; Chakhmakhcheva, O. G.; Ovchinnikov, Y. A. *Nucl. Acids Res.* **1983**, *11*, 8369 - 8387) as well as reaction of the 5'-hydroxyl group of the support-bound oligonucleotide analog with bis(β-cyanoethoxy)-*N,N*-diisopropylphosphite and subsequent oxidation with *tert*-butyl hydroperoxide (Horn, T.; Urdea, M. S. *DNA* **1986**, *5*, 421 - 426).

15 After purification by polyacrylamide gel electrophoresis, oligonucleotide analogs **I** - **V** were obtained in >95% purity in all five cases as determined by capillary gel electrophoresis. Analysis of the purified products by MALDI-TOF mass spectrometry confirmed the expected molecular weights (Pieles, U.; Zürcher, W.; Schär, M.; Moser, H. *Nucleic Acids Res.* **1993**, *21*, 3191 - 3196). **I**: calc. mass: 4109 , found: 4112. **II**: calc.: 4243, found: 4244. **III**: calc.: 4272, found: 4281. **IV**: calc.: 4232, found: 4231.

16 The lack of hysteresis is indicative of the absence of triple helix formation, as the isosequential PNA showed a large hysteresis in thermal melting experiments. K.-H. Altmann, D. Hüsken, unpublished results.