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Mixed Oligonucleotide Analogues with an Acyclic Carbohydrate Moiety and a *N*-Cyanoguanidine Functionality

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Abstract : *Mixed oligonucleotide analogues having a backbone structure with a N-cyanoguanidine functionality and an acyclic sugar moiety were synthesized. This combination, however, has a detrimental effect on duplex stability of DNA-DNA hybrids.*

The synthesis of acyclic oligonucleotide analogues with phosphodiester internucleotide linkages has been discouraged because of reports on the absence of duplex formation between acyclic oligothymidylates and natural oligoadenylates[1]. A recent report, however, describes the potential advantages of acyclic oligonucleotide analogues : stabilisation against enzymatic degradation, triple helix formation of the acyclic polyadenylates with natural oligothymidylates and their use as universal nucleosides for the synthesis of degenerate probes[2].

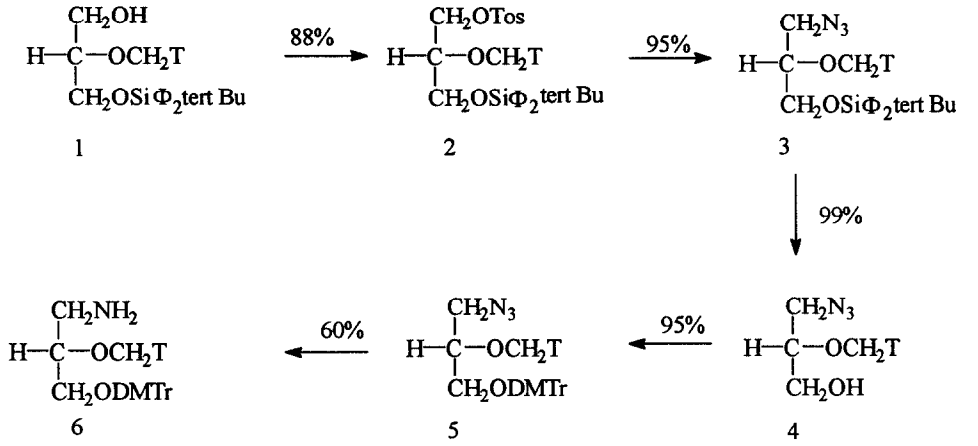
The observation of Nielsen *et al.*[3] that peptide nucleic acids (PNA) having an *N*-(2-aminoethyl)glycine backbone are able to hybridize strongly with the complementary natural oligonucleotide in a sequence specific manner[4] increased the interest in the synthesis of acyclic oligonucleotides with an amide-containing backbone[5].

In a follow up of our recent work on *N*-substituted guanidines as neutral backbones in oligonucleotides[6], we became interested in the synthesis of acyclic oligonucleotide analogues having this functionality. With these syntheses, we decided to further exploit the beneficial characteristics of these *N*-substituted guanidines functionalities: a planar geometry, restricted rotation, nuclease stability and good hybridization properties of oligonucleotides having this backbone structure.

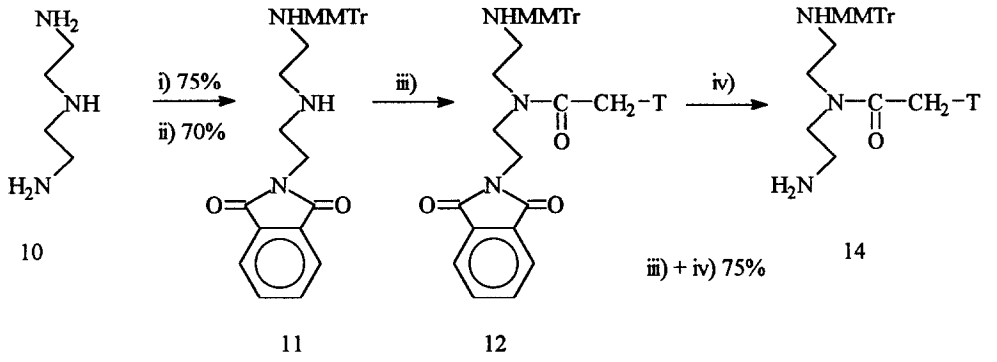
Here we describe the synthesis and physicochemical characteristics of oligonucleotides where the natural deoxyribose moiety is partially replaced by acyclic nucleosides of different chain length and where the phosphate backbone is partially replaced by the substituted guanidine moiety. The thymine base is connected to the backbone either by an ether function either by an amide function.

The synthesis of the starting material **1**, is essentially that described by Azymah et al.[7]. Tosylation of **1** with tosylchloride in pyridine afforded **2** which was reacted with sodium azide in DMF for 4 hours at 80°C. The silyl protecting group was removed with tetrabutylammonium fluoride in THF for 1 hour at room

temperature, yielding **4** which was dimethoxytritylated (DMTrCl, pyridine). The azido group of **5** was reduced under catalytic conditions (H_2, Pd/C).

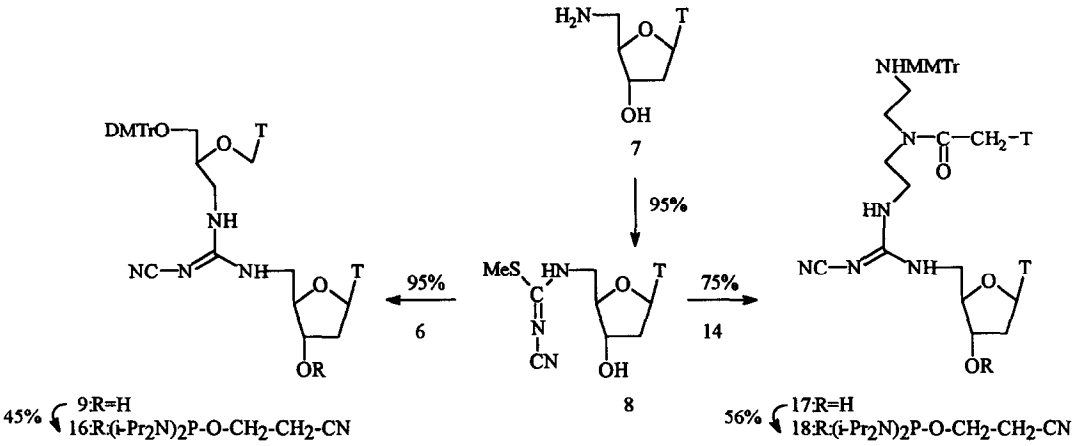


Reaction of 5'-amino-5'-deoxythymidine **7** [8] with *S,S*-dimethyl-*N*-cyanodithioimidocarbonate in ethanol at room temperature afforded 1-cyano-3-(5'-deoxythymidin-5'-yl)-2-methylisothiourea **8**. Reaction of **8** with **6** in a mixture of DMF:TEA (1:1) containing $AgNO_3$ (1 1 equiv.) afforded dimer **9**.



Reaction of **8** with **14** using identical reaction circumstances as described for the synthesis of **9** yielded dimer **17**. The starting material **14** was obtained from diethylenetriamine **10** by monomethoxytritylation (5 eq. of **10** in pyridine), protection of the other primary amine function as a phtalimide (N-carbethoxyphtalimide, EtOAc, H_2O, NaOH; ΔT, toluene), introduction of the methylenecarbonyl linked thymine and removal of the phtalimide protecting group with hydrazine.

The dimers **9** and **17** were phosphitylated with 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite and the oligonucleotides were assembled on an automated DNA synthesizer (ABI 381A) and were purified on a Mono Q® HR 10/10 column (Pharmacia).



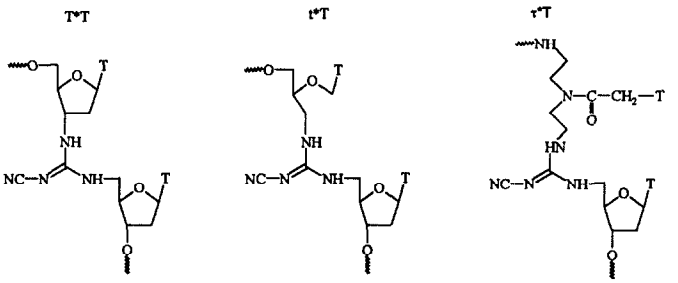
Duplex stability of oligonucleotide analogues containing dimer **9** (t*T) and **17** (τ*T) were compared with natural oligonucleotides having a normal phosphodiester backbone and with oligonucleotides where only the internucleotide linkage (cyanoguanidine backbone) is modified.

An oligonucleotide with one or two cyanoguanidine linkages gives only a slight decrease in melting point (entries 1, 2, 5) and even the presence of eight modified internucleotide linkages yields an oligonucleotide which is still able to hybridize with its complementary sequence (entries 8, 9).

However, the introduction of an acyclic nucleoside has a more detrimental influence on duplex stability which can be seen from the large drop in T_m, by introducing one or two of these constructs (entries 3, 4, 6, 7) and by the absence of hybridization by introducing five cyanoguanidine bearing acyclic nucleosides (entry 10). As could be expected, increasing the chain length did not alter the melting point behaviour. (entry 4)

Melting points were determined with $(dA)_{13}$ or $(dA)_{17}$ as complementary strand at 4μM each, in a 0.02 M phosphate buffer pH 7.5 containing 0.1 M NaCl and 0.1 mM EDTA..

entry	oligonucleotide 5'→3'	T_m°C	ΔT_m
1	T_{13}	33.0	
2	$T_5(T^*T)T_6$	29.8	- 3.2
3	$T_6(t^*T)T_5$	22.7	-10.3
4	$T_6(\tau^*T)T_5$	22.6	-10.4
5	$(T^*T)T_8(T^*T)T$	31.2	- 1.8
6	$(t^*T)T_8(t^*T)T$	23.5	- 7.7
7	$T_8(t^*T)(t^*T)T$	15.1	-16.1
8	T_{17}	43.0	
9	$(T^*T)_8T$	24.0	-19.0
10	$T_2(t^*T_pT)_5$	-	-



Therefore, it can be concluded that this new internucleotide linkage with restricted rotation is not able to compensate for the loss in entropy on duplex formation between oligonucleotides containing acyclic nucleosides and natural oligonucleotides. As also demonstrated by the synthesis of mixed oligonucleotides containing acyclic and pyranose nucleosides[2], it will not be easy to construct a mixed oligonucleotide, differently modified at several positions, with good hybridization properties.

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