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Synthesis and Stability of Oligonucleotides Containing Acyclic Achiral Nucleoside Analogues with Two Base Moieties

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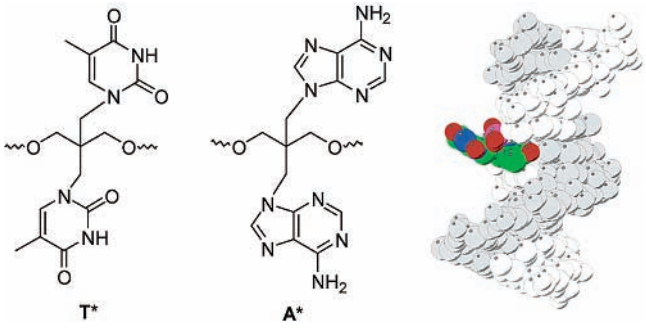
ABSTRACT



Nucleotide building blocks with two base moieties were synthesized and incorporated into oligonucleotides. One of the two bases is involved in base pairing within the double helix, while the other base is sticking out of the minor groove. This system may be used for presenting sequence information at the outside of the helix.

The double-stranded DNA consists of two phosphorylated deoxyribose backbones on which purine and pyrimidine bases are attached, base pairing with each other. The sequence of bases constitutes the informational part of DNA. As demonstrated by Watson and Crick,[1] this genetic information is pointed inward to the double helix and unwinding is needed to make this information available (for replication and transcription). However, for building less complicated systems (compared to the biological organization of a cell), it would be more straightforward when this information can be used more directly, i.e., when the bases are pointing outside the helix. DNA consists of three moieties (phosphate, sugar, base), and all three of them can be used for presenting information outside the helix. The internucleotide phosphate group has been used intensively to attach reporter groups[2] or cationic residues[3] or to be replaced by other information-containing functionalities.[4] Also the sugar moiety has been presented outside the helix structure by using disaccharide nucleotide units.[5] Here, we present the synthesis of a double-headed oligonucleotide that can be used to present sequence information at the outside of the double helix (Figure 1).

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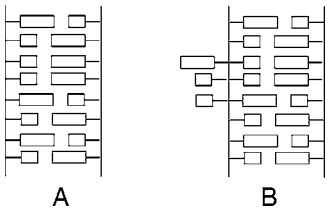


Figure 1. Picture of the Watson−Crick double helix (A) and of a duplex containing double-headed DNA (B).

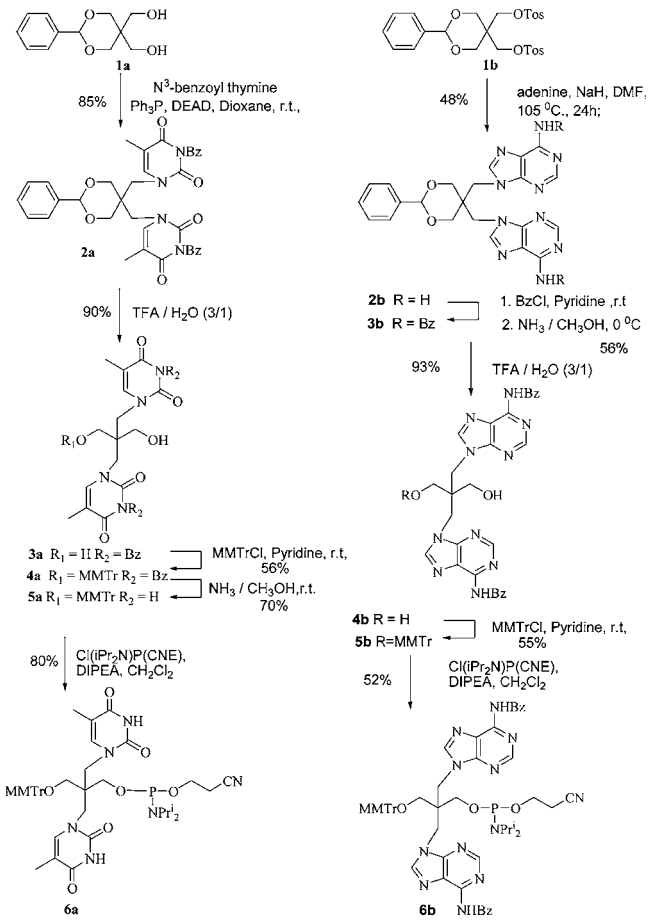
The synthesis and hybridization properties of the first example of double-headed DNA are described.

Oligonucleotides containing acyclic nucleoside analogues usually bind poorly to DNA and RNA as a result of loss in entropy upon duplex formation, caused by the flexibility of the acyclic units.[6] Despite this, we preferred to use an acyclic nucleotide as our first model. Indeed, we do not have information on the geometry that is needed for a nucleotide containing two base moieties to fit in a helix structure. To start with a simple model, we also decided to synthesize a symmetric nucleoside building block (i.e., with identical bases) having two primary hydroxyl groups (to avoid much steric hindrance for the tritylation and phosphoramidite synthesis and to avoid the chirality problem). This is exemplified by the nucleosides 2,2-bis[(thymin-1-yl)methyl]-propane-1,3-diol (T*) and 2,2-bis[(adenin-9-yl)methyl]-propane-1,3-diol (A*).

The synthetic route to 1-*O*-monomethoxytrityl-2,2-bis-[(thymin-1-yl)methyl]-1,3- propanediol phosphoramidite **6a** and 1-*O*-monomethoxytrityl-2,2-bis[(adenin-9-yl)methyl]-1,3-propanediol phosphoramidite **6b** is outlined in Scheme 1. The 2-phenyl-5,5-bis(hydroxyl methyl)-1,3-dioxane **1a** was synthesized according to the procedure described previously.[7] Treatment of **1a** under Mitsunobu conditions with Ph_3P, N^3-benzoylthymine, and diethyl azodicarboxylate (DEAD) in THF offered 2-phenyl-5,5-bis(thymin-1-yl)-1,3-dioxane **2a** in 85% yield. The benzylidene protecting group of **2a** was removed using TFA/H_2O (3/1), giving **3a** in 90% yield, followed by tritylation with MMTrCl in dry pyridine to afford **4a** in 56% yield. Removal of the benzoyl protecting group was achieved by treatment of compound **4a** with saturated NH_3/MeOH to give **5a** in 70% yield. Finally, phosphitylation of compound **5a** gave the desired phosphoramidite **6a** in 80% yield.

Compound **1a** was converted to **1b** as described previously.[8] The two tosyloxy groups of **1b** were substituted with

Scheme 1. Synthesis of Nucleoside Phosphoramidite Building Blocks



adenine using NaH and adenine in dry DMF, giving **2b** in 48% yield. Protection of the exocyclic amino group of the adenine base was achieved using excess benzoyl chloride in pyridine. Subsequent treatment with saturated NH_3/MeOH at 0 °C for 30 min resulted in the desired monobenzoyl-protected amines **3b** in 56% yield. Removal of the benzylidene group of **3b** by treatment with TFA/H_2O (3/1) afforded **4b** in 93% yield, which was reacted with MMTrCl in anhydrous pyridine to give **5b** in 55% yield. Finally, phosphitylation of compound **5b** gave the desired phosphoramidite **6b**, in a somewhat lower yield of 52%.

With the phosphoramidites **6a** and **6b** of the building blocks T* and A* at hand, several modified oligonucleotides were synthesized comprising single or multiple bis-thyminyl or bis-adeninyl nucleotides. The thermal stability of these oligonucleotides with either DNA or RNA as complement was investigated. The results for the influence of incorporation of one modified T* and one modified A* on duplex stability of a mixed dsDNA sequence are shown in Table 1. Likewise, the influence of mismatches opposite T* and A* on the thermal stability of the same duplex is shown.

Compared to unmodified DNA, incorporation of the modified nucleotide T* or A* in the middle of the oligonucleotides led to a decrease in T_m of 5.9 or 8.0 °C, respectively (entries 2 and 7). At first sight, this decrease in

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Table 1. Influence of Incorporation of One Modified Nucleotide (A* or T*) on dsDNA Stability and Their Mismatch Discrimination Properties

sequences d(3′-GGTCACXATACG-5′)
d(5′-CCAGTGYTATGC-3′)

entry	X−Y base pair	T_m (°C)[a]	ΔT_m (°C)	entry	X−Y base pair	T_m (°C)[a]	ΔT_m (°C)
1	T−A	49.1		6	A −T	49.4	
2	T*−A	43.2	−5.9	7	A*−T	41.4	−8.0
3	T*−T	34.1	−15.0	8	A*−A	35.3	−14.1
4	T*−G	36.0	−13.1	9	A*−G	36.3	−13.1
5	T*−C	29.1	−20.0	10	A*−C	31.3	−18.1

[a] T_m was determined at UV 260 nm in NaCl (0.1 M), KH_2PO_4 (20 mM, pH 7.5), EDTA (0.1 mM), with 4 μM of each strand.

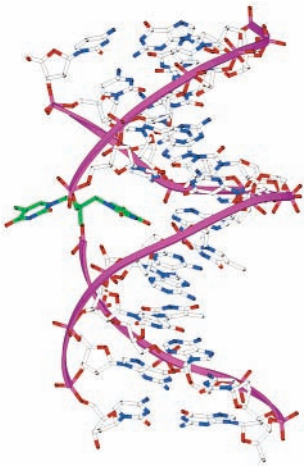

Figure 2. The average structure obtained from the simulation. The average structure was calculated from the last 50 ps of a 500-ps MD simulation. Figure created with Molscript.

melting point may lead to the conclusion that no base pairing occurs at the modified site. The mismatch sequences are considerably less stable, however, and a ΔT_m ranging from −13.1 to −20.0 °C is observed. This means that base pair discrimination is still present and that the T−A base pair is more stable than the mismatches.

To visualize the potential interaction of the modified nucleotide at the insertion site, a model was built. The duplex used for model building has the sequence 5′-(GCATAT*-CACTGG)-3′ with its complement, with T* representing the modified nucleotide. The final 50 ps were used to calculate an average structure (Figure 2).

A hydrogen bond analysis does not give an indication of broken or missing hydrogen bonds in the duplex. However, the higher flexibility of the T* residue might destabilize the duplex and act as a nucleus of disorder in the duplex when heating the system, resulting in a lower melting temperature compared to that of the unmodified duplex.

The hybridization properties of oligonucleotides, containing more than one successive modification, toward complementary DNA are shown in Table 2. This experiment is carried out with the aim of determining if the presence of more sequence information outside the helix will not lead to unacceptable duplex destabilization.

Increasing the number of A* nucleotides within an oligonucleotide results in further reduction of absolute binding affinity. However, the ΔT_m mod^{-1} decreases going from one to four incorporated A* moieties (incorporation of one A* causes relative more destabilization than three or four A* ΔT_m mod^{-1}, which is demonstrated with different sequences). This might suggest that some stacking interaction between the extra helical adenine bases of A* is possible. It should be mentioned that this effect is not seen with T*, i.e., a pyrimidine base that is known to demonstrate less stacking properties and that gives more steric hindrance at the N^1 position, giving a pyrimidine base less opportunity for rotation around the C1′−N^1 bond.

Hybridization of modified oligonucleotides with complementary RNA was studied likewise (Table 3). For oligonucleotides containing modified thymine or adenine nucleotides (T* or A*), the ΔT_m mod^{-1} decreases as the number of modified thymidine or adenine nucleotides (T* or A*) increases (up to five T* and three A* were evaluated). The oligonucleotide with three successive T* displayed a T_m of 40.8 °C (entry 20). Upon incorporation of five T* monomers, the T_m (entry 21) versus the RNA complement increased to 47.7 °C, almost identical to the T_m of the duplex with only one modified T* modification (entry 19). Notably, the T_m

Table 2. Influence of Incorporation of Several Modified Nucleotides (A*) on the Stability of Deoxyoligonucleotides Hybridized to Their DNA Complement

entry	sequences	T_m (°C)[a]	ΔT_m mod^{-1} (°C)	mass calcd[b]	ESI-MS
11	d(5′-CGGCAAAAACGCC-3′)	59.1			
12	d(5′-CGGCAAA*AACGCC-3′)	50.8	−8.3	4054.8	4055.3
13	d(5′-CGGCAA*A*A*ACGCC-3′)	43.9	−5.1	4292.9	4293.4
14	d(5′-CGGCAAAAAACGCC-3′)	60.8			
15	d(5′-CGGCAAA*A*AACGCC-3′)	49.3	−5.7	4486.9	4487.3
16	d(5′-CGGCAA*A*A*A*ACGCC-3′)	44.7	−4.0	4725.0	4725.5
17	d(5′-CGGCA*A*A*A*A*A*CGCC-3′)	36.3	−4.0	4963.1	4963.7

[a] T_m was determined at UV 260 nm in NaCl (0.1 M), KH_2PO_4 (20 mM, pH 7.5), EDTA (0.1 mM), with 4 μM of each strand. [b] Calculated monoisotopic mass.

Table 3. Influence of Incorporation of Several Modified Nucleotides (A* or T*) on the Stability of Deoxyoligonucleotides Hybridized to Their RNA Complement

entry	sequences	T_m (°C)[a]	ΔT_m mod^{-1} (°C)	mass calcd[b]	ESI-MS
18	d(5′-GGCGTTTTTGCCG-3′)	56.7			
19	d(5′-GGCGTTT*TTGCCG-3′)	48.7	−8.0	4080.7	4081.2
20	d(5′-GGCGTT*T*T*TGCCG-3′)	40.8	−5.3	4300.8	4301.3
21	d(5′-GGCGT*T*T*T*T*GCCG-3′)	47.7	−1.8	4520.9	4521.4
22	d-AAAAAAAAAAAAA	14.5			
23	d-AAAAAAA*AAAAAA	13.3	−1.2	4126.9	4127.6
24	d-AAAAAA*A*A*AAAAA	15.1	+0.2	4365.0	4365.9

[a] T_m was determined at UV 260 nm in NaCl (0.1 M), KH_2PO_4 (20 mM, pH 7.5), EDTA (0.1 mM), with 4 μM of each strand. [b] Calculated monoisotopic mass.

for the oligonucleotide with three A* modifications is 0.6 °C higher than for the unmodified duplex (entry 24), although we deal here with a different model [oligo (dA): oligo U] of low stability.

In conclusion, novel oligonucleotides containing 2,2-bis-[(thymin-1-yl)methyl]-propane-1,3-diol and 2,2-bis[(adenin-9-yl)methyl]-propane-1,3-diol were assembled on an automated DNA synthesizer. Oligonucleotides with one acyclic achiral nucleoside (A* or T*) incorporated in the middle of a 12-mer or 13-mer show decreased hybridization properties with complementary DNA or RNA. Likewise, oligonucleotides comprising several consecutive acyclic achiral nucleosides (A*) in the middle show pronounced reduction in duplex stability for their complementary DNA. The ΔT_m mod^{-1}, however, decreases with the incorporation of successive modification. Using RNA as the complement, the duplexes are much more stable, and in some cases, even an increase in duplex stability is observed. Acyclic oligonucleotides consisting entirely of 2,2-bis[(thymin-1-yl)methyl]-propane- 1,3-diol were likewise synthesized. The duplex with complementary DNA or RNA proved unstable.

Overall we could demonstrate that stable double-stranded DNA can be obtained presenting base moieties outside the helix. These results encourage us to evaluate in the future the stability of new double-headed DNA with the same and different bases attached to a sugar or sugarlike motif instead of the acyclic motif and to investigate the way this information can be used.

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Supporting Information Available: Experimental procedures and spectral data for compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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