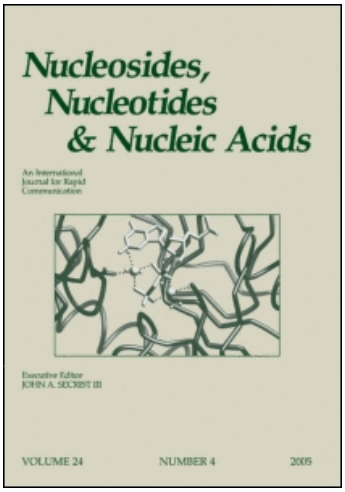

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Hoogsteen-Duplex DNA: Synthesis and Base Pairing of Oligodeoxynucleotides Containing 1-Deaza-2'-deoxyadenosine

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HOOGSTEEN-DUPLEX DNA: SYNTHESIS AND BASE PAIRING OF OLIGODEOXYNUCLEOTIDES CONTAINING 1-DEAZA-2'-DEOXYADENOSINE

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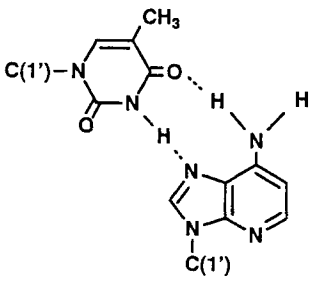
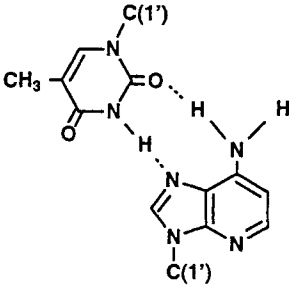
ABSTRACT. Solid-phase synthesis of oligonucleotides containing 1-deazaadenine was carried out employing phosphonate and phosphoramidite chemistry. Hoogsteen base pairing was established for the duplex $d(c^1A_{20})\cdot d(T_{20})$.

Hoogsteen base pairing is the common binding mode for the third strand of triplex DNA. This type of base pairing has also been suggested for homopolyribonucleotide duplexes carrying bulky substituents at position 2 of the adenine moiety[1]. Also a non-Watson-Crick duplex of poly(c^1A) with poly(U) has been described[2]. Recently, single crystal X-ray analyses have shown that the ambiguous base pairing of dI with dA or dG occurs by the Hoogsteen mode within regular Watson-Crick oligonucleotides[3]. A parallel-stranded Hoogsteen duplex of oligonucleotides of a special sequence has been described which is stable in acidic medium.[4] This manuscript[5] is the first report on a Hoogsteen duplex DNA formed under neutral conditions. The duplex is constructed from two oligonucleotide strands, one containing 1-deaza-2'-deoxyadenosine (**1a**) and the other 2'-deoxythymidine.



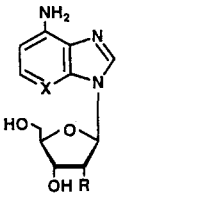
Hoogsteen base pair



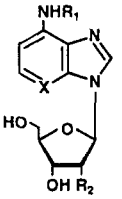
reverse Hoogsteen base pair

The oligonucleotide building blocks **4a** and **5a** as well as their ribofuranosyl counterparts **4b** and **5b** were synthesized. Also the phosphoramidite **5c** derived from

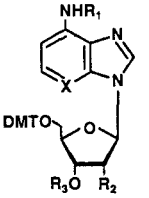
1,3-dideaza-2'-deoxyadenosine (**1c**) was prepared. The 1-deaza-2'-deoxyadenosine (**1a**) was obtained from the nitro nucleoside[6]; compound **1b** was prepared according to Mizuno[7], the 1,3-dideaza derivative **1c** has been described recently[8]. Compound **1a** was protected with a benzoyl residue (**2a**: m.p. 127-128°C, 77%), a methoxyacetyl group was used for **1b** (**2b**: m.p. 194°C, 86%), and a fmoc group for **1c** (**2c**: m.p. 146-147°C, 76%). The half-lifes (conc. aq. ammonia) are the following: **2a** (125 min; 50°C); **2b** (17 min; 40°C); **2c** (8 min, 50°C). The 5'-OH groups were blocked by the 4,4'-dimethoxytrityl residue under standard conditions (**3a**: 76%; **3b**: 88%; **3c**: 79%). Silylation of **3b** with $(i\text{-}Pr)_3SiCl$ ($AgNO_3$) afforded **3d** (76%) and **3e** (14%). The reaction with tris(1,2,4-triazoyl)phosphite furnished the phosphonates **4a** and **b** (**4a**: ^{31}P-NMR: 2.56 (^{1}J(P,H)) = 587 Hz, ^{3}J (P,3'-H) = 8.3 Hz; **4b**: 2.28 (^{1}J(P,H)) = 594 Hz, ^{3}J (P,3'-H) = 9.3 Hz). The ß-cyanoethylphosphoramidites **5a** (^{31}P-NMR: 148.2, 148.8), **5b** (149.2, 152.2), and **5c** (149.4, 149.6) were also synthesized as well as the polymer-linked **6a-c**.



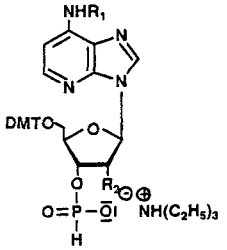
1a: X = N; R = H
1b: X = N; R = OH
1c: X = CH; R = H



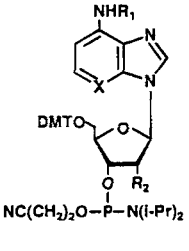
2a: X = N; R_1 = bz; R_2 = H
2b: X = N; R_1 = mac; R_2 = OH
2c: X = CH; R_1 = fmoc; R_2 = H



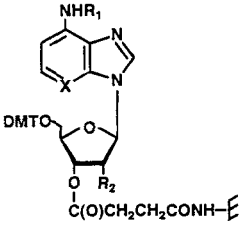
3a: X = N; R_1 = bz; R_2 = H; R_3 = H
3b: X = N; R_1 = mac; R_2 = OH; R_3 = H
3c: X = CH; R_1 = fmoc; R_2 = H; R_3 = H
3d: X = N; R_1 = mac; R_2 = OSi(iPr)$_3$; R_3 = H
3e: X = N; R_1 = mac; R_2 = OH; R_3 = OSi(iPr)$_3$



4a: R_1 = bz; R_2 = H
4b: R_1 = mac; R_2 = OH



5a: X = N; R_1 = bz; R_2 = H
5b: X = N; R_1 = mac; R_2 = OH
5c: X = CH; R_1 = fmoc; R_2 = H



6a: R_1 = bz; R_2 = H
6b: R_1 = mac; R_2 = OH
6c: X = CH; R_1 = fmoc; R_2 = H

The ^{13}C-NMR-data of the 1-deazaadenine ribonucleosides are summarized in Table 1.

The building blocks **4a**, **4b**, and **5a-c** were utilized in solid-phase synthesis. Oligoribo- and oligodeoxyribonucleotides were prepared.

Table 1. ^{13}C-NMR chemical shifts of **1b**, **3b**, **4b**, **5b**, **6b-d** measured in [D_6]DMSO.

Compd.	C-2	C-3a	C-5	C-6	C-7	C-7a	
1b	140.0	146.5	144.2	102.4	147.4	123.8	
2b	142.7	146.9	145.3	107.0	136.3	125.5	
3b	142.5	147.0	145.5	107.1	136.1	125.3	
3c	142.8	147.0	145.4	107.0	136.1	125.4	
3d	143.2	149.7	146.7	106.9	136.0	125.4	
4b	142.4	147.0	145.2	106.9	136.0	125.3	
6b	143.4	146.2	145.3	107.1	136.2	125.3	
Compd.	C-1'	C-2'	C-3'	C-4'	C-5'	CO	OMe
1b	88.7	72.9	71.1	86.2	62.1	-	-
2b	88.1	73.5	70.7	85.7	61.7	169.3	58.9
3b	88.2	73.0	70.5	83.1	63.9	169.3	58.9
3c	88.4	74.4	70.7	83.9	63.6	169.3	58.8
3d	88.5	72.1	72.2	83.6	63.3	169.1	58.7
4b	87.4	73.3	73.1	83.8	63.5	169.1	58.7
6b	87.0	74.3	69.9	82.6	62.1	169.1	58.7

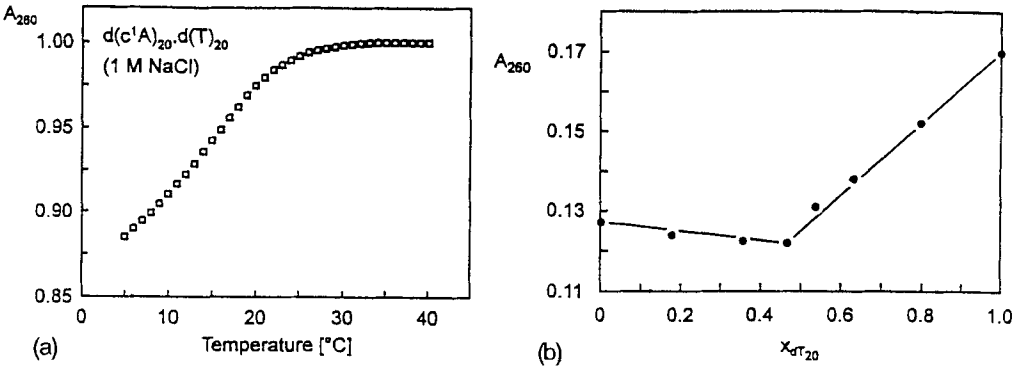


FIGURE 1

A_5-c^1A_2-A_5 (**7**) U_{12} (**8**) $d(T_{20})$ (**9**) $d(c^1A_{20})$ (**10**)
$d(c^1A_{10}$-$T_{10})$ (**11**) $d(T_{10}$-$c^1A_{10})$ (**12**) $d(c^1c^3A_{20})$ (**13**)

The oligoribonucleotide A_5-c^1A_2-A_5 (**7**) was hybridized with U_{12} (**8**) (**7·8**: T_m = 20°; $U_{12}{\cdot}A_{12}$: T_m = 24°). The duplex destabilization can be explained by the imperfect hydrogen bonding of the modified base or Hoogsteen base pairing. In the case of the complex $d(c^1A_{20}){\cdot}d(T_{20})$, a cooperative melting profile was observed (Figure 1a) resulting in a T_m-value of 15°C (60 mM Na-cacodylate, 1 M NaCl, 100 mM $MgCl_2$, pH 7.0). The duplex formation was also confirmed by the mixing profile shown in Fig. 1b. The equimolar mixture of $d(c^1c^3A_{20})$ and $d(T_{20})$ which was prepared by phosphoramidite chemistry did not form a Hoogsteen duplex.

In the case of the duplex **9·10**, parallel or antiparallel strand-orientation as well as Hoogsteen or reverse Hoogsteen base pairing have to be considered. Nevertheless, the melting experiments carried out on the block oligomers **11** and **12** showed parallel strand-orientation.

In principle the duplex of $d(c^1A_{20}){\cdot}d(T_{20})$ can be formed by Hoogsteen or reverse Hoogsteen base pairing. However, in the light of the large number of data available for homopyrimidine-homopurine-homopyrimidine triplex structures the duplex **9·10** is expected to show Hoogsteen base pairing.

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