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Stability and structural features of the duplexes containing nucleoside analogues with a fixed N-type conformation, 2'-*O*,4'-*C*-methyleneribonucleosides

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Abstract

Bicyclic nucleoside analogues with a fixed N-type conformation, 2'-*O*,4'-*C*-methyleneuridine and -cytidine, were incorporated into oligonucleotides, and the binding efficiency of the modified oligonucleotides to the complementary DNA and RNA as well as the CD spectra of the modified DNA-DNA and modified DNA-RNA duplexes were studied.

Keywords: Nucleic acid analogues; Nucleosides; Oligomers; Circular dichroism

Extensive efforts have been directed toward developing novel DNA and RNA analogues for the practical use of antisense and antigene technologies [1-5]. In these studies, several types of conformationally restricted nucleoside analogues were synthesized by our laboratory [6,7] and other groups [8]. In the previous paper [6], we reported the first synthesis of bicyclic nucleoside analogues with a fixed N-type conformation, 2'-*O*,4'-*C*-methyleneuridine **1a** and -cytidine **1b**.[1] It is well known that the duplex formation of the preorganized oligonucleotides (ONs) with complementary DNA and RNA are entropically favorable [11]. In addition, for the reason that the A-form RNA duplex possesses the N-type sugar conformation while the B-form DNA duplex possesses the S-conformation [12], the nucleoside analogues with a rigid N-type conformation, such as **1**, are readily expected to enhance the hybridization ability towards complementary RNA. Here, we wish to demonstrate the duplex stability of the ONs containing the conformationally fixed nucleoside analogues **1**, and the structural features of these duplexes.

As shown in Scheme 1, the phosphoramidite building block **3** was prepared from the corresponding 5'-*O*-dimethoxytrityl derivative **2** [6] by the usual method [13]. The modified ONs **5-13** as well as unmodified RNA and DNA strands **16-20** were synthesized by standard

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1 Just after our publication of the synthesis of **1** [6], Wengel and co-workers reported the synthesis and some properties of the same compounds [9,10].

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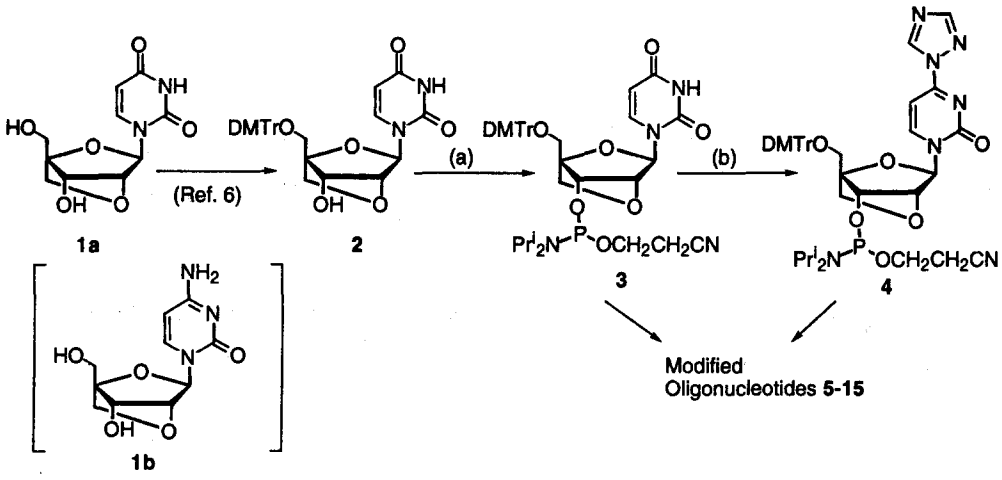


Scheme 1. (a) 2-cyanoethyl *N,N,N',N'*-tetraisopropylphosphordiamidite, diisopropylammonium tetrazolide, MeCN-THF (3:1), r.t., 1.5 h, 81%; (b) 1,2,4-triazole, $POCl_3$, Et_3N, MeCN, 0 °C, 4 h, 89%.

Table 1
Tm Values (°C) of the modified ONs **5-12** towards complementary RNA **17** and DNA **18**. [a]

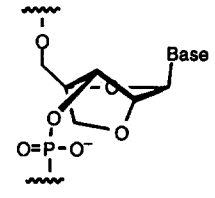
Oligonucleotides		RNA Complement **17** 5'-r (AGCAAAAAACGC)-3'	DNA Complement **18** 5'-d (AGCAAAAAACGC)-3'
5'-d (GCGTTTTTTGCT)-3'	**16**	45	47
5'-d (GCGXTTTTTGCT)-3'	**5**	49 (+4)	50 (+3)
5'-d (GCGTTXTTTGCT)-3'	**6**	49 (+4)	49 (+2)
5'-d (GCGTTTXTTGCT)-3'	**7**	50 (+5)	49 (+2)
5'-d (GCGTTTTTXGCT)-3'	**8**	51 (+6)	52 (+5)
5'-d (GCGXXTTTTGCT)-3'	**9**	53 (+4)	51 (+2)
5'-d (GCGTTXXTTGCT)-3'	**10**	53 (+4)	50 (+1.5)
5'-d (GCGTTTTXXGCT)-3'	**11**	55 (+5)	54 (+3.5)
5'-d (GCGXXXXXXGCT)-3'	**12**	71 (+4.3)	58 (+1.8)

a. Duplex concentration: 4 μM. Buffer: 100 mM NaCl,10 mM sodium phosphate buffer (pH 7.2); The values in parentheses are ΔTm/modifications.

Table 2
Tm Values (°C) of the modified ONs **13-15** towards complementary DNA **20**. [a]

Oligonucleotides		DNA Complement **20** 5'-d (AAAAGGGAGAGAGA)-3'
5'-d (TCTCTCTCCCTTTT)-3'	**19**	46
5'-d (XCXCXCXCCCXXXT)-3'	**13**	54 (+1.1)
5'-d (TYTYTYTYYYTTTT)-3'	**14**	65 (+3.2)
5'-d (XYXYXYXYYYXXXT)-3'	**15**	74 (+2.2)

a. See footnote in Table 1.



phosphoramidite protocol on the DNA synthesizer.[2] In addition, the modified ONs **14** and **15**, in which 2'-*O*,4'-*C*-methylenecytidine **1b** was introduced, were prepared by the post-elongation-modification procedure [14]. The phosphoramidite **3** was treated with triazole, $POCl_3$ and Et_3N to afford the triazolouridine derivative **4** in good yield, which can be converted into the cytidine derivative by treatment with conc. ammonia after ON synthesis.[2]

The binding efficiency of the modified ONs to the complementary sequences was assessed by an analysis of the UV melting curve. The melting temperatures (Tm) for the modified ONs **5-12** and **13-15** are summarized in Tables 1 and 2, respectively. Incorporation of the nucleoside analogues **1** into ONs significantly enhances hybridization ability towards the complementary strand. Especially, in the case of the modified ONs **5-12**, the remarkable thermal stability of the duplexes with complementary RNA **17** was observed (ΔTm/modifications towards **17** = *ca.* +5 °C, ΔTm/modifications towards **18** = *ca.* +3 °C), which was derived from the rigid N-form structure of nucleoside analogues **1**.

In order to investigate a thermodynamic contribution of the conformationally restricted nucleoside analogue to duplex stability, the parameters ($\Delta H°$, $\Delta S°$ and $\Delta G°_{37°C}$) for the duplexes **16•17** and **12•17** were determined by van't Hoff plots [15]. The results are shown in Table 3. The free energy ($\Delta G°_{37°C}$) of the modified duplex **12•17** is increased when compared with that of the unmodified DNA-RNA duplex **16•17**. Furthermore, it is noteworthy that the modified duplex **12•17** showed a quite favorable $\Delta S°$, relative to the unmodified duplex. From these results, the difference in binding ability towards complementary RNA between the modified ON **12** and the unmodified one **16** (ΔTm = +26 °C) translates to a difference in binding entropy ($\Delta\Delta S°$) of *ca.* 28 cal•mol^{-1}•K^{-1}.

Table 3

Thermodynamic parameters for duplex formation between modified ON 12 and Complementary RNA 17. [a]

Duplexes	$\Delta H°$ (kcal • mol^{-1})	$\Delta S°$ (cal • mol^{-1} • K^{-1})	$\Delta G°_{37°C}$ (kcal • mol^{-1})
16 • 17	-98.38	-282.0	-10.95
12 • 17	-96.98	-254.2	-18.18

a. Thermodynamic parameters were determined in 10 mM sodium phosphate buffer (pH 7.2) with 100 mM NaCl. Duplex concentrations ranged from 0.5 to 6.0 μM.

CD spectra of the modified (**12•18**) and unmodified DNA-DNA duplexes **16•18** were measured as well as those of the modified (**12•17**) and unmodified DNA-RNA duplexes **16•17**, which indicated the structural features of the modified duplexes (Fig. 1). The CD spectrum of the modified DNA-DNA duplex **12•18** had a relatively strong positive band at 271 nm and a relatively weak negative band at 245 nm, while the spectrum of the unmodified duplex **16•18** had roughly equal intensity of positive and negative bands at 283 and 250 nm, respectively. Similar tendency was observed in the spectrum of the modified DNA-RNA duplex **12•17**. The strong positive band for the modified duplex **12•17** was shifted to shorter wave lengths, and the

[2] The modified ONs were synthesized on the DNA synthesizer (Gene Assembler® Plus, Pharmacia, 0.2 μmol scale, 5'-dimethoxytrityl on). After treatment with conc. ammonia, removal of the 5'-dimethoxytrytyl group and purification were performed on NENSORB™ PREP reversed-phase columns. The purity of the modified ONs was verified using reversed-phase HPLC and the compositions were determined by MALDI-MS.

negative band around 240 nm was reduced in comparison with these bands in the spectra of unmodified duplex **16•17**. These observations supported that the incorporation of the nucleoside analogue **1a** into ONs transform the duplexes into A-like conformation.

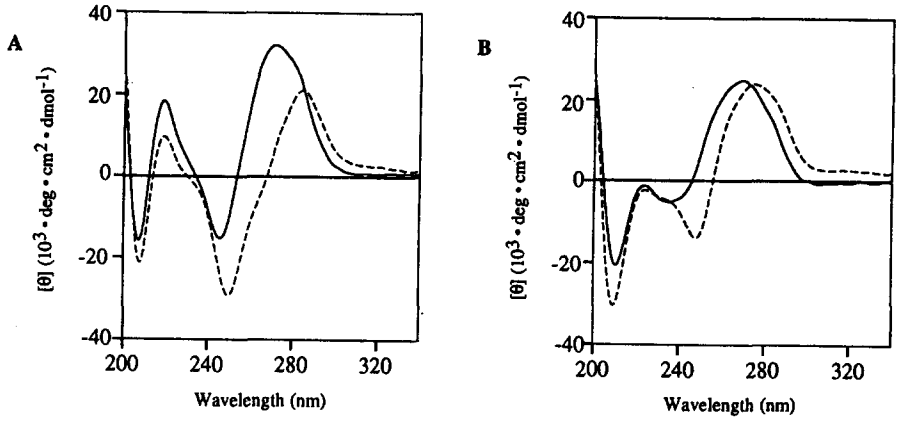


Fig. 1. CD Spectra of the modified and unmodified duplexes. A, modified DNA-DNA duplex **12•18** (solid line); unmodified duplex **16•18** (dashed line). B, modified DNA-RNA duplex **12•17** (solid line); unmodified duplex **16•17** (dashed line). Duplex concentration: 4 μM. Buffer: 100 mM NaCl, 10 mM sodium phosphate buffer (pH 7.2): Temp 18 °C.

We have demonstrated here that the conformationally restricted nucleoside analogues **1** have a significant hybridization ability with complementary nucleic acids, especially with complementary RNA, which arises from the entropically favorable character of **1**. These results should reveal a promising route to development of antisense methodology.

Acknowledgements

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References

[1] Uhlmann E, Peyman A. Chem. Rev. 1990;90:543-584.
[2] Beaucage SL, Iyer RP. Tetrahedron 1993;49:6123-6194.
[3] Milligan JF, Matteucci MD, Martin JC, J. Med. Chem. 1993;36:1923-1937.
[4] Thoung NT, Hélène C. Angew. Chem., Int. Ed. Engl. 1993;32:666-690.
[5] Hyrup B, Nielsen PE. Bioorg. Med. Chem. 1996;4:5-23.
[6] Obika S, Nanbu D, Hari Y, Morio K-i, In Y, Ishida T, Imanishi T. Tetrahedron Lett. 1997;38:8735-8738.
[7] Obika S, Morio K-i, Nanbu D, Imanishi T. Chem. Commun. 1997:1643-1644.
[8] Herdewijn P. Liebigs Ann. Chem. 1996:1337-1348.
[9] Singh SK, Nielsen P, Koshkin AA, Wengel J. Chem. Commun. 1998:455-456.
[10] Koshkin AA, Singh SK, Nielsen P, Rajwanshi VK, Kumar R, Meldgaard M, Olsen CE, Wengel J. Tetrahedron 1998;54:3607-3630.
[11] Kool ET. Chem. Rev. 1997;97:1473-1487.
[12] Saenger W. Principles of Nucleic Acid Structure. New York: Springer-Verlag, 1984.
[13] Sinha ND, Cook RM. Nucleic Acids Res. 1988;16:2659-2669.
[14] Scherr M, Klebba C, Häner R, Ganser A, Engels JW. Bioorg. Med. Chem. Lett. 1997;7:1791-1796.
[15] Borer PN, Dengler B, Tinoco I, Uhlenbeck OC. J. Mol. Biol. 1974;86:843-853.