Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 515–518

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

PREPARATION AND PROPERTIES OF 2',5'-LINKED OLIGONUCLEOTIDE ANALOGUES CONTAINING 3'-*O*,4'-*C*-METHYLENERIBONUCLEOSIDES

Satoshi Obika, Ken-ichiro Morio, Yoshiyuki Hari, and Takeshi Imanishi*

Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, Osaka 565-0871, Japan.

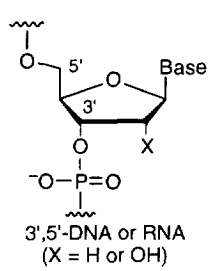
Received 28 October 1998; accepted 22 December 1998

Abstract: Bicyclic nucleoside analogues, 3'-*O*,4'-*C*-methyleneuridine and -5-methyluridine, were successfully incorporated into oligonucleotides *via* connection with 2',5'-phosphodiester linkage, and hybridization behavior and nuclease stability of the modified oligonucleotides were investigated.

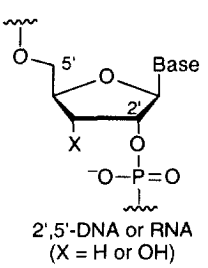
Keywords: Nucleic acids; Nucleotides; Nucleosides; DNA

Various oligonucleotide modifications have been developing in recent years because of their vast potential for diagnostic and therapeutic agents.[1-5] Since the first reports about the properties of 'non-genetic' 2',5'-linked oligonucleotides (2',5'-DNA or 2',5'-RNA) in 1992,[6-8] intensive studies on 2',5'-DNA or -RNA have been carried out which revealed that the hybridization affinity of 2',5'-DNA or -RNA/3',5'-RNA is somewhat inferior to that of the 3',5'-DNA/3',5'-RNA duplex, although 2',5'-DNA or -RNA shows binding selectivity for 3',5'-RNA over 3',5'-ssDNA.[9-13]

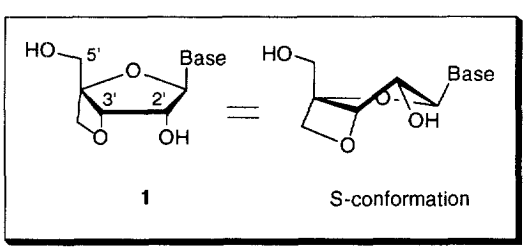
Recently, we have reported the synthesis and conformational analysis of the bicyclic nucleoside analogues, 3'-*O*,4'-*C*-methyleneribonucleosides **1**, potential synthons for 2',5'-linked oligonucleotide modifications.[14] Judging from ^{1}H NMR analysis and PM3 calculation, the bicyclic nucleosides **1** were found to exist predominantly in S-conformation, while 3'-deoxyribonucleosides have mainly N-conformation and natural ribonucleosides exist in equilibrium between S- and N-conformations.[14,15] Therefore, it is of great interest to study the relationship between nucleoside conformation and some properties, such as hybridization ability, of 2',5'-linked oligonucleotides containing the bicyclic nucleosides **1**.



3',5'-DNA or RNA
(X = H or OH)



2',5'-DNA or RNA
(X = H or OH)



1 S-conformation

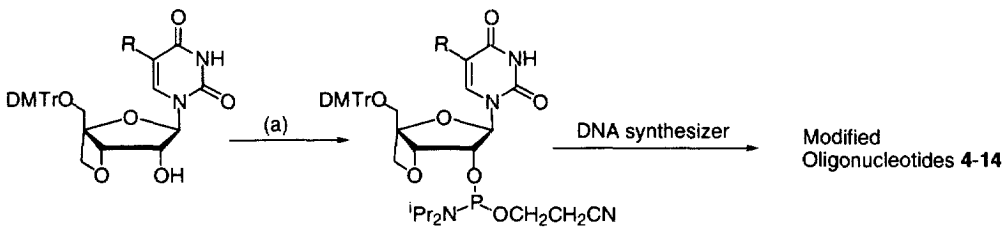
* e-mail: imanishi@phs.osaka-u.ac.jp

PII: S0960-894X(99)00028-1

In this paper, we describe the synthesis of 2',5'-linked oligonucleotide modifications containing the bicyclic nucleoside analogues, 3'-*O*,4'-*C*-methyleneuridine and -5-methyluridine, their hybridization properties, and the resistant ability to the snake venom phosphodiesterase (SVPDE).

As shown in Scheme 1, the phosphoramidite building blocks **3** were prepared from the corresponding 5'-*O*-dimethoxytrityl derivatives **2**[14,16] by the usual method.[17] The modified oligonucleotides **4-14** were successfully synthesized using the standard phosphoramidite protocol on the DNA synthesizer.[18]

Scheme 1



2a (R = H)
2b (R = Me)

3a (R = H)
3b (R = Me)

(a) 2-Cyanoethyl *N,N,N',N'*-tetraisopropylphosphordiamidite, diisopropylammonium tetrazolide, MeCN-THF (3:1), r.t., 30 min, 82-100%.

The binding efficiency of the modified oligonucleotides to the complementary DNA or RNA in 10 mM sodium phosphate buffer, pH 7.2, and 100 mM sodium chloride was assessed by an analysis of the UV melting curve. The melting temperatures (*T*m) for the modified oligonucleotides **4-13** are summarized in Table 1 which shows that the modified oligonucleotides **4-12** gave little depression or some increase in *T*m towards complementary RNA **16** as compared with the unmodified oligonucleotide **15** (ΔTm/modifications = -1.3 to +2 for **4a-12a**, and -1 to +4 for **4b-12b**), while moderate depressions in *T*m were observed towards complementary ssDNA **17** (ΔTm/modifications = -6.5 to 0 for **4a-12a**, and -5.5 to 0 for **4b-12b**).[19] The oligonucleotides **8-13** with two or more modifications showed distinct difference between the RNA case and the DNA case in ΔTm/modifications. Especially, the oligonucleotide **13a** with six modifications maintained its hybridization ability towards complementary RNA **16**, in spite of the lack of hybridization ability of **13a** towards complementary DNA **17**. No significant difference in the hybridization ability was observed between the modified oligonucleotides **4a-12a** series and **4b-12b** series containing 3'-*O*,4'-*C*-methyleneuridine and 3'-*O*,4'-*C*-methylene-5-methyluridine, respectively. These results clearly indicate that the modified oligonucleotides **4-13** containing the conformationally restricted nucleoside analogues **1** bind with complementary RNA more strongly than 2',5'-RNA does,[9,13] and also have significant selectivity for RNA over ssDNA as well as 2',5'-DNA or -RNA.[9-13] The details are not clear; however, these results are probably ascribable to preorganization of the modified oligonucleotides in suitable conformation[20] together with 2',5'-phosphodiester linkages.

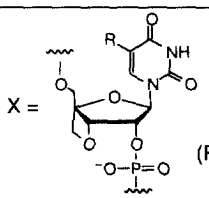
As shown in Figure 1, the stability of the modified oligonucleotide **14a** to SVPDE, one of the most representative 3'-exonucleases, was also investigated by monitoring the change in absorbance at 260 nm. The unmodified and 3'-end modified oligonucleotides **18** and **14a** were treated with SVPDE at 37 °C. The half-lives

of the modified oligonucleotide **14a** was estimated to be *ca.* 1400 sec while that of unmodified oligonucleotide **18** was *ca.* 350 sec. The partial digestion of the modified oligonucleotide **14a** was observed which, however, was mainly due to its slow endonucleolytic activity rather than exonucleolytic activity.[13] Therefore, the modified oligonucleotide **14a** was shown to be more stable than the unmodified oligonucleotide **18**.

Table 1
*T*m Values (°C) of the modified oligonucleotides **4-13** towards complementary RNA **16** and DNA **17**.[a]

Oligonucleotides		RNA Complement **16** 5'-r (AGCAAAAAACGC)-3'		DNA Complement **17** 5'-d (AGCAAAAAACGC)-3'	
		a (R = H)	**b** (R = Me)	**a** (R = H)	**b** (R = Me)
5'-d (GCGTTTTTTGCT)-3'	**15**	45		47	
5'-d (GCGXTTTTTGCT)-3'	**4**	45 (±0)	46 (+1)	47 (±0)	44 (-3)
5'-d (GCGTTXTTTGCT)-3'	**5**	47 (+2)	47 (+2)	47 (±0)	47 (±0)
5'-d (GCGTTTXTTGCT)-3'	**6**	44 (-1)	47 (+2)	44 (-3)	46 (-1)
5'-d (GCGTTTTTXGCT)-3'	**7**	47 (+2)	49 (+4)	43 (-4)	47 (±0)
5'-d (GCGXXTTTTGCT)-3'	**8**	43 (-1)	46 (+0.5)	34 (-6.5)	36 (-5.5)
5'-d (GCGTTXXTTGCT)-3'	**9**	44 (-0.5)	44 (-0.5)	37 (-5)	38 (-4.5)
5'-d (GCGTTTTXXGCT)-3'	**10**	43 (-1)	43 (-1)	36 (-5.5)	40 (-3.5)
5'-d (GCGXTTTTXGCT)-3'	**11**	46 (+0.5)	ND[b]	37 (-5)	ND[b]
5'-d (GCGXTXTXTGCT)-3'	**12**	41 (-1.3)	42 (-1)	30 (-5.7)	33 (-4.7)
5'-d (GCGXXXXXXGCT)-3'	**13**	30 (-2.5)	ND[b]	<5[c] (<-7)	ND[b]

a. Duplex concentration, 4 μM; buffer, 100 mM NaCl,10 mM sodium phosphate buffer (pH 7.2); the values in parentheses are Δ*T*m/modifications.
b. Not determined.
c. Obvious thermal hypochromicity was not observed.

X = 

(R = H or Me)

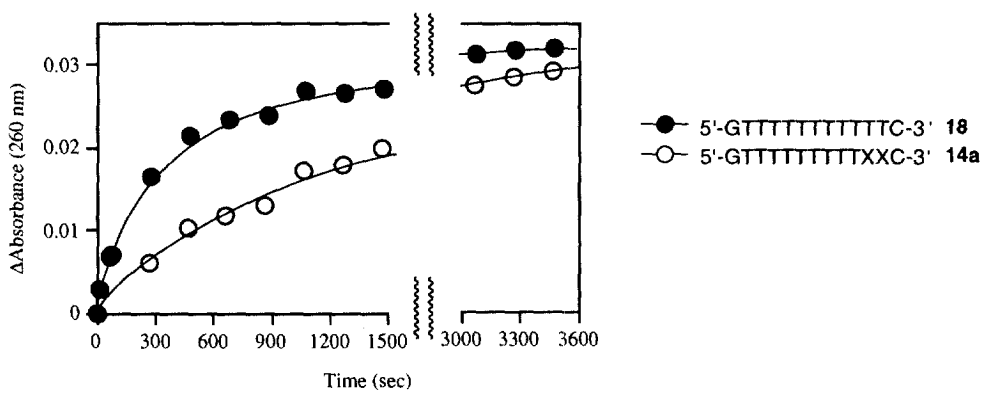


Figure 1. Time course of SVPDE digestion of oligonucleotides **14a** and **18**.
Oligonucleotide concentration, 4 μM; enzyme, 0.0012 U snake venom phosphodiesterase (Böehringer); buffer, 100 mM NaCl, 14 mM $MgCl_2$, 100 mM Tris-HCl (pH 8.6); temp., 37 °C.

Thus, we have demonstrated here that the modified oligonucleotides with the conformationally restricted nucleoside analogues **1** have favorable hybridization ability towards complementary RNA with significant selectivity for RNA over ssDNA. Moreover, incorporation of the nucleoside analogues **1** into the 3'-end of the oligonucleotide was shown to enhance the expected nuclease resistance. These features mean that the 2',5'-linked oligonucleotide modifications with the bicyclic nucleoside analogues **1** are a promising candidate for antisense methodology.

Acknowledgements

A part of this work was supported by a Grant-in Aid for Scientific Research, No. 09557201 and No. 09772025 from the Ministry of Education, Science, Sports and Culture, Japan.

References and Notes

1. Uhlmann, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 543.
2. Beaucage, S. L.; Iyer, R. P. *Tetrahedron* **1993**, *49*, 6123.
3. Milligan, J. F.; Matteucci, M. D.; Martin, J. C. *J. Med. Chem.* **1993**, *36*, 1923.
4. Thoung, N. T.; Hélène, C. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 666.
5. Hyrup, B.; Nielsen, P. E. *Bioorg. Med. Chem.* **1996**, *4*, 5.
6. Kierzek, R.; He, L.; Turner, D. H. *Nucleic Acids Res.* **1992**, *20*, 1685.
7. Dougherty, J. P.; Rizzo, C. J.; Breslow, R. *J. Am. Chem. Soc.* **1992**, *114*, 6254.
8. Hashimoto, H.; Switzer, C. *J. Am. Chem. Soc.* **1992**, *114*, 6255.
9. Giannaris, P. A.; Damha, M. J. *Nucleic Acids Res.* **1993**, *21*, 4742.
10. Alul, R.; Hoke, G. D. *Antisense Res. Dev.* **1995**, *5*, 3.
11. Prakash, T. P.; Jung, K.-E.; Switzer, C. *Chem. Commun.* **1996**, 1793.
12. Sheppard, T. L.; Breslow, R. *J. Am. Chem. Soc.* **1996**, *118*, 9810.
13. Kandimalla, E. R.; Manning, A.; Zhao, Q.; Shaw, D. R.; Byrn, R. A.; Sasisekharan, V.; Agrawal, S. *Nucleic Acids Res.* **1997**, *25*, 370.
14. Obika, S.; Morio, K.; Nanbu, D.; Imanishi, T. *Chem. Commun.* **1997**, 1643.
15. In recent years, various kinds of conformationally restricted (or fixed) nucleosides (or nucleotides) were prepared and their properties were reported: Herdewijn, P. *Liebigs Ann. Chem.* **1996**, 1337 and references cited therein; Zou, R.; Matteucci, M. D. *Tetraahedron Lett.* **1996**, *37*, 941; Obika, S.; Nanbu, D.; Hari, Y.; Morio, K.; In, Y.; Ishida, T.; Imanishi, T. *Tetrahedron Lett.* **1997**, *38*, 8735; Obika, S.; Nanbu, D.; Hari, Y.; Andoh, J.; Morio, K.; Doi, T.; Imanishi, T. *Tetrahedron Lett.* **1998**, *39*, 5401; Singh, S. K.; Nielsen, P.; Koshkin, A. A.; Wengel, J. *Chem. Commun.* **1998**, 455; Koshkin, A. A.; Singh, S. K.; Nielsen, P.; Rajwanshi, V. K.; Kumar, R.; Meldgaard, M.; Olsen, C. E.; Wengel, J. *Tetrahedron* **1998**, *54*, 3607.
16. The synthesis of 5'-*O*-dimethoxytrityl-3',*O*-4,*C*-methylene-5-methyluridine (**2b**) will be reported elsewhere.
17. Sinha, N. D.; Cook, R. M. *Nucleic Acids Res.* **1988**, *16*, 2659.
18. The oligonucleotides were synthesized on DNA synthesizer (Gene Assembler® Plus, Pharmacia, 0.2 μmol scale, 5'-dimethoxytrityl on). After cleavage from the support and deprotection with conc. ammonia at 70 °C for 3 h, removal of the 5'-*O*-dimethoxytrityl group and purification of the oligonucleotides were performed on NENSORB™ PREP reverse-phase columns. The purity of these oligonucleotides was verified using analytical HPLC and the composition was determined by MALDI-TOF-mass spectrometry. UV-Spectroscopically determined yields after chromatographic purification were in the range of 6-40%.
19. From the *T*m measurements targeting mismatched DNA sequences, recognition by the modified oligonucleotides containing **1** was found to be sequence selective. For example, the *T*m value of the modified oligonucleotide **5a** towards its mismatched sequence, 5'-d(AGCAAATAACGC)-3' **19**, was 33 °C, while that of the unmodified oligonucleotide **15** towards **19** was 37 °C.
20. Kool, E. T. *Chem. Rev.* **1997**, *97*, 1473.