Pergamon

Tetrahedron Letters 41 (2000) 221–224

TETRAHEDRON LETTERS

Triplex formation by an oligonucleotide containing conformationally locked *C*-nucleoside, 5-(2-*O*,4-*C*-methylene-β-D-ribofuranosyl)oxazole

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Received 8 September 1999; revised 25 October 1999; accepted 29 October 1999

Abstract

The triplex-forming ability of oligonucleotide analogues containing conformationally locked *C*-nucleosides, 5-(2-*O*,4-*C*-methylene-β-D-ribofuranosyl)oxazole or its 2-phenyl congener, towards a purine sequence of duplex DNA with a single C·G base pair interruption is studied.

Keywords: nucleic acid analogues; molecular recognition; bicyclic heterocyclic compounds; oxazoles.

Pyrimidine oligonucleotides can bind to homopurine tracts of duplex DNA in a sequence-specific manner to form triple-helical structures through the formation of Hoogsteen hydrogen bonds.[1] These triplex-forming oligonucleotides (TFOs) have received a considerable amount of attention as novel agents to control specific gene expression (the so-called antigene strategy).[2] However, the duplex DNA recognized by TFOs is limited to homopurine sequences, and pyrimidine nucleotide interruptions in the homopurine sequences reduce the triplex stability.[3] Although it is known that guanine can interact with a T·A base pair to form a G·T·A triad and that thymine can also form a T·C·G triad through a single hydrogen bond,[4] the stabilizing effect by these natural base triads is insufficient. To overcome this problem, some oligonucleotide modifications in the nucleobase moiety have been reported to date.[2,5] However, a practical method for the recognition of general duplex DNA sequences containing all four base pairs is still lacking.

In order to design and synthesize a nucleobase analogue to accomplish efficient recognition of a pyrimidine·purine base pair, it must be taken into consideration that the hydrogen bond donors and acceptors should be favorably located to form proper hydrogen bonds and that the phosphate backbone should coincide in geometry with that of a pyrimidine·purine·pyrimidine triad. From our modeling study, it occurred to us that the five-membered heterocycles, such as oxazole and imidazole, would be the most potent candidate for recognition of a C·G base pair through triplex formation.

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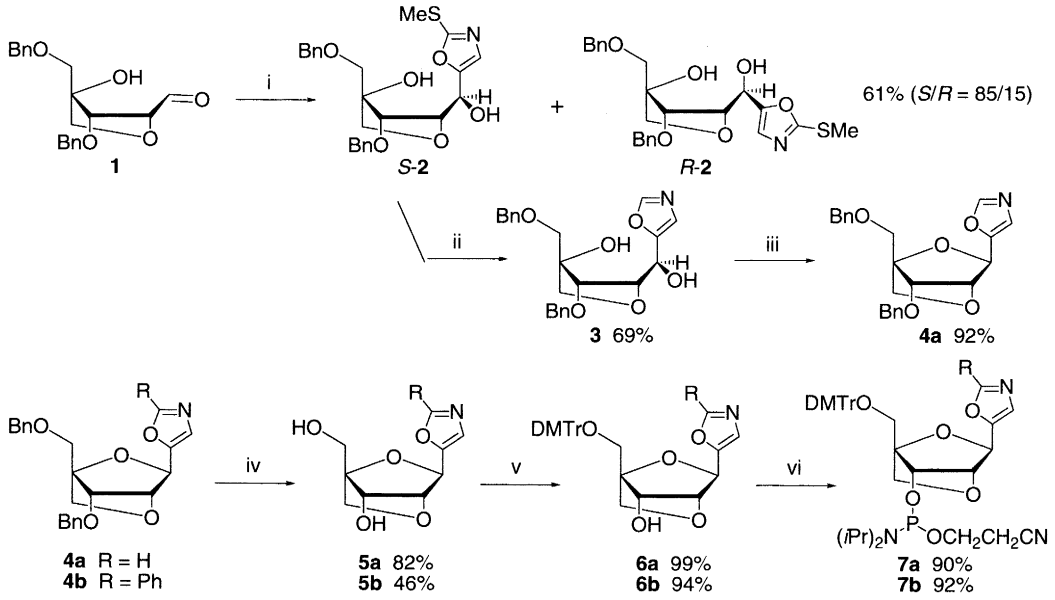
0040-4039/00/$ - see front matter
PII: S0040-4039(99)02052-3

Conformationally locked oligonucleotides containing 2′-*O*,4′-*C*-methyleneribonucleosides are of great interest because of their potent hybridizing ability towards complementary ssDNA or RNA, due to their entropically favorable character.[6] Here we describe the recognition of a C·G base pair in a homopurine DNA sequence by an oligonucleotide containing conformationally locked *C*-nucleoside, 5-(2-*O*,4-*C*-methylene-β-D-ribofuranosyl)oxazole.

As shown in Scheme 1, conformationally locked *C*-nucleoside, 5-(3,5-*O*-dibenzyl-2-*O*,4-*C*-methylene-β-D-ribofuranosyl)oxazole **4a**, was synthesized according to the previously reported procedure.[7] The aldehyde **1** was treated with 2-methylthio-5-oxazolylmagnesium bromide to afford the desired *S*-epimer of **2** as a main product (61%, *S:R*=85:15). The methylthio group in *S*-**2** was removed (*S*-**2**→**3**, 69%) and the subsequent ring-closure reaction gave the β-anomer of **4a** exclusively (92%). Hydrogenolysis of **4a** and its 2-phenyl congener **4b**[7] afforded the diols **5a** and **5b** (82 and 46%), respectively. The phosphoramidites **7a** and **7b**, the suitable building blocks for DNA synthesis, were obtained via dimethoxytritylation (**5**→**6**, 94–99%) and phosphitylation (**6**→**7**, 90–92%). Modified TFOs **I** (X=**8** and **9**) containing conformationally locked *C*-nucleosides were effectively prepared by standard phosphoramidite protocol on a DNA synthesizer.†



Scheme 1. Reagents and conditions: (i) 2-methylthio-5-oxazolylmagnesium bromide (4 equiv.), THF, rt; (ii) Raney-Ni, EtOH, reflux; (iii) 1,1′-azobis(*N*,*N*-dimethylformamide), tributylphosphine, benzene, rt; (iv) H_2, $Pd(OH)_2$-C, EtOH, rt; (v) DMTrCl, pyridine, rt; (vi) 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite, diisopropylammonium tetrazolide, MeCN–THF, rt

Measurements of melting temperature (*T*m) were carried out in 7 mM sodium phosphate buffer (pH 6.0) containing 140 mM potassium chloride and 0.5 mM magnesium chloride.‡ The melting profile for **I**·**II**·**III** (**8**·C·G) triplex in which TFO **I** contains 5-(2-*O*,4-*C*-methylene-β-D-ribofuranosyl)oxazole is

† The modified oligonucleotides 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 oligonucleotides was verified using reversed-phase HPLC and the compositions were determined by MALDI-MS.

‡ According to the literature,[8] the buffer for *T*m measurements which approximates the intracellular cationic environment and the sequences of the target duplex are selected.

shown in Fig. 1, and all *T*m data of TFO dissociation are summarized in Table 1. Triplex formation between **I** (X=**8**) and **II**·**III** (Y·Z) is sequence-selective, and the binding strength of the **8**·C·G triad is preferable to that of T·C·G and other triads.[§] On the other hand, TFO containing 2-phenyloxazole congener **9** exhibits weaker binding affinity for all four base pairs.[¶] Such differences in *T*m values between TFOs **I** (X=**8**) and **I** (X=**9**) suggest that the hydrogen bonding interaction between the 3-nitrogen of the oxazole moiety in **8** and the 4-amino group hydrogen in cytidine is essential for stabilizing the triplex, and that the interaction is obstructed by the steric hindrance of 2-phenyl group in **9** as illustrated in Fig. 2.

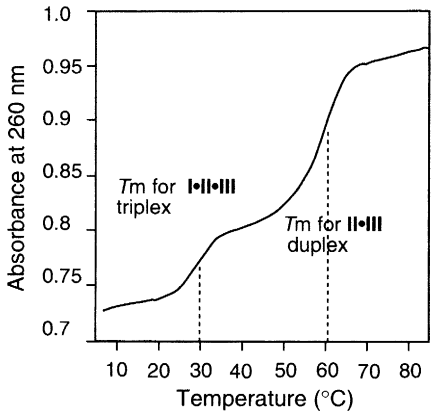


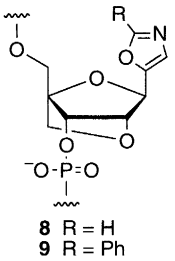
Fig. 1. Melting profile of **I**·**II**·**III** (**8**·C·G) triplex

Table 1
*T*m values (°C) of **I**·**II**·**III** (**X**·**Y**·**Z**) triplexes

5'-d(TTTTTCT**X**TCTCTCT)-3' **I**
5'-d(GCTAAAAAGA**Y**AGAGAGATCG)-3' **II**
3'-d(CGATTTTTCT**Z**TCTCTCTAGC)-5' **III**

	Y•Z			
X	C•G	G•C	T•A	A•T
8	**30**	22	26	21
9	23	18	25	23
A	23	29	19	25
G	24	26	30	18
C	26	50	16	18
T	27	21	17	44

Conditions: 7 mM sodium phosphate buffer (pH 6.0), 140 mM KCl, 0.5 mM $MgCl_2$, [oligonucleotide] = 1.5 μM for each strand.



8 R = H
9 R = Ph

§ In addition to the results in Table 1, we also elucidated the triplex-forming ability of another TFO 5′-d(TTTTT^{m}CT**X**T^{m}CT^{m}CT^{m}CT)-3′ (**IV**), in which cytosine bases are replaced by 5-methylcytosine (^{m}C), towards the target duplex 5′-d(GCTAAAAAGA**C**AGAGAGTCG)-3′ (**II**)/3′-CGATTTTTCT**G**TCTCTCTAGC)-5′ (**III**). The *T*m measurements were carried out under neutral conditions [7 mM sodium phosphate buffer (pH 7.0), 140 mM potassium chloride and 10 mM magnesium chloride] and the *T*m value of **IV** (X=**8**)·**II**·**III** was found to be 29°C, while that of **IV** (X=A, G, ^{m}C or T)·**II**·**III** was 21, 20, 25 or 25°C, respectively.

¶ This result is in fair agreement with that of the oligonucleotide containing 1-(2-deoxy-β-D-ribofuranosyl)-4-phenylimidazole, reported by Dervan et al.[9]

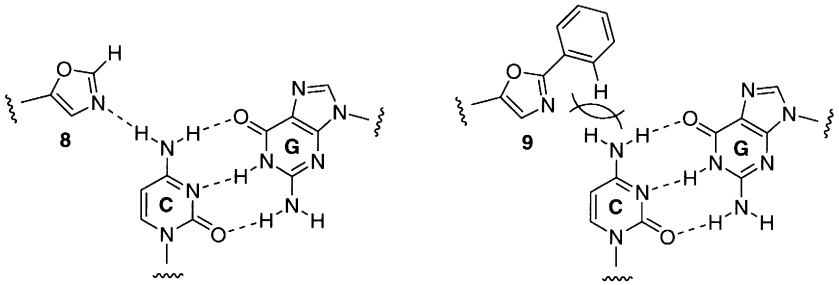


Fig. 2. Proposed hydrogen bonding scheme for **8**·C·G and **9**·C·G triads

These results indicate that the conformationally locked *C*-nucleoside, 5-(2-*O*,4-*C*-methylene-β-D-ribofuranosyl)oxazole can recognize a C·G base pair in a homopurine DNA sequence through triplex formation. Although the structure of **8** is simple and only one hydrogen bonding is proposed between **8** and a C·G base pair, the binding ability of **8** towards a C·G base pair seems to be comparable to that of N^4-(6-amino-2-pyridinyl)deoxycytidine, which is one of the most suitable compounds for C·G base pair recognition, reported by Miller et al.[10] This favorable feature of **8** may be due to its conformationally locked structure. Thus, this type of *C*-nucleoside analogue would be a usable synthon for novel and effective antigene oligonucleotides.

Acknowledgements

Part of this work was supported by a grant-in-aid for Scientific Research (B), no. 09557201, from Japan Society for the Promotion of Science. We are also grateful to the Takeda Science Foundation for financial support.

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