Pergamon

Tetrahedron Letters 39 (1998) 6167–6170

TETRAHEDRON LETTERS

Incorporation of Serinol Derived Acyclic Nucleoside Analogues into Oligonucleotides: Influence on Duplex and Triplex Formation

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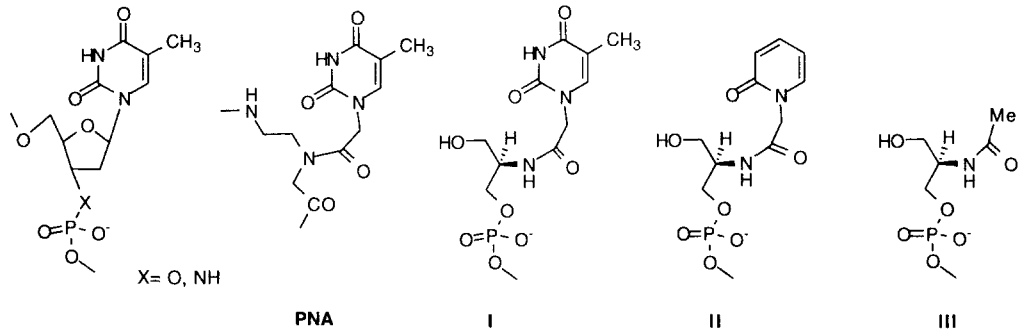
Received 13 May 1998; accepted 13 June 1998

Abstract : Phosphoramidites **6a-c** were readily prepared by an original route and used to incorporate serinol derived units **I-III** in oligonucleotides which, compared to their natural analogues, exhibited a decreased hybridization capacity for duplex and triplex formation.

Oligopyrimidines (Y) can recognize oligopyrimidine•oligopurine (Y•R) sequences in double-stranded DNA (dsDNA) by triple helix formation[1]. In these systems, recognition and sequence specificity are mainly achieved by means of Hoogsteen hydrogen bonding between the bases of the third strand and hydrogen donor or acceptor sites on the oligopurine strand in the major groove of the duplex, although other factors such as base stacking and electrostatic interactions must also be taken into account. Unfortunately, such DNA triplexes generally exhibit weaker thermodynamic stability than the corresponding DNA duplexes. Accordingly, it has been proposed to tether or insert various intercalating agents at the ends[2] of or within[3] the triplex-forming oligonucleotides (TFOs) in order to increase their binding strength to target duplex sequences.



Scheme 1: Nucleotide analogues

Another approach being currently evaluated, aims at modifying deoxyribose phosphodiester backbone. The development of peptide nucleic acids (PNAs) has so far represented the most significant progress (Scheme 1). PNAs are characterized by a pseudo peptide-backbone which confers to these analogues an unprecedented capacity to bind not only to the complementary single-stranded DNA or RNA but also to ds-DNA by forming a 2:1 complex (triplex) with the oligopurine strand and by displacing the oligopyrimidine strand which is initially engaged in Watson-Crick base pairing with the oligopurine sequence.[4] This remarkable behaviour (P-loop

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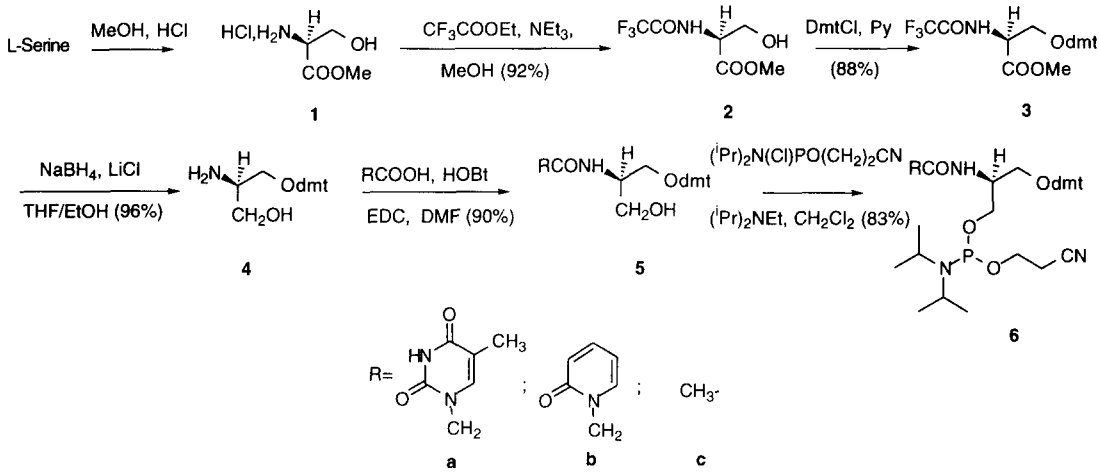
0040-4039/98/$ - see front matter
PII: S0040-4039(98)01268-4

formation) was not observed with other phosphodiester backbone analogues such as methylphosphonates and phosphorothioates which failed to form stable triple helix in Hoogsteen configuration.[5,6] In contrast, another oligonucleotide analogue in which the phosphodiester bonds were replaced by N3' → P5' phosphoramidates exhibited a remarkable ability to form a stable triple helice with its DNA target.[7]

Along this line of research, we have been interested at evaluating the effects of the incorporation of an acyclic analogue of the natural deoxynucleosides within a TFO. Moreover, although oligonucleotides containing acyclic nucleosides form Watson-Crick base paired duplexes with decreased stability as compared to natural oligonucleotides[8], acyclic residues remain highly promising as they might be used to incorporate into synthetic oligonucleotides modified base analogues or stabilizing agents.[9]

In this communication, we propose a new convenient synthetic pathway[10] to readily obtain serinol derived units **I-III** (Scheme 1), which can be incorporated by the phosphoramidite approach into synthetic oligonucleotides. Furthermore, we have examined the binding capacity of these oligonucleotides containing serinol derived acyclic nucleoside analogues towards their complementary targets either by Watson-Crick (duplex) or by Hoogsteen base pairing (triplex).



Scheme 2

Thus, the appropriately functionalized derivative of serinol **4** was obtained in good overall yield, starting from *L*-serine methyl ester **1**, by following the straightforward route depicted in Scheme 2. Indeed, treatment of a methanolic solution of **1** with ethyl trifluoroacetate provided the *N*-trifluoroacetate **2** which was subsequently dimethoxytritylated to give the fully protected derivative **3**. Reduction-deprotection of the ester and amino functions of **3** was accomplished by reaction with lithium borohydride which produced the amino-alcohol **4**. This readily available compound can now serve to attach at the amino position various derivatives of interest for hybridization (duplex and triplex) studies.

3' **A A GA A G A A A AAA G A** 5'	**7**
5' **T T C T T C T T Z T T T C T** 3'	**8**
5' **A C A G T T A A GA A G AA Y AA A G A T T G A C G** 3'	**9**
3' **T G T C A A T T C T T C T T X T T T C T A A C T C G** 5'	**10**

Compound **4** was first used to introduce N^1-thyminylacetic acid to obtain **5a** which, after treatment with cyanoethyl diisopropylchlorophosphoramidite, gave **6a**. This phosphoramidate was used to incorporate

the corresponding acyclic nucleoside analogue **I** at position 9 (in place of Z) of the 14-mer oligonucleotide **8** (**Z= I**). The latter was prepared and purified by using the standard protocol for oligonucleotide synthesis. Similarly, thymine was replaced by 2-pyridone (which is likely unable to establish any appropriate hydrogen bonding but could still provide stacking interaction with neighbouring bases) and by a simple acetyl group (which exhibits further reduced hydrogen bonding and stacking capacity), using phosphoramidites **6b** and **6c** to obtain oligonucleotides **8** (**Z= II**) and **8** (**Z= III**), respectively.

Z	Thymidine	**I** (Thymine)	**II** (2-Pyridone)	**III** (Acetyl)
Tm (± 1°C)	47	35	30	30

Table 1 : 20 mM cacodylate, 100 mM NaCl, 10 mM $MgCl_2$, pH6

We first investigated the thermal stability of the duplexes formed between oligonucleotides **7•8**, and compared the melting temperature (*Tm*) of the unmodified oligonucleotide **8** (Z=Thymidine) with that of their acyclic nucleoside analogues **8** (Z=**I**), **8** (Z=**II**) and **8** (Z=**III**), respectively, in which the position Z was substituted by a serinol unit being either tethered to a thymine (**I**), a 2-pyridone (**II**) or a simple acetyl group (**III**). Table 1 shows that the introduction of **I** decreased the stability of the duplex (ΔTm = -12 °C), whereas a similar substitution by a pyridone (**II**) or an acetyl group (**III**) resulted in a further decreased stability (ΔTm = -17 °C).

Tm **(+/- 1°C)**

X . Y **Z**	**T . A**	**A . T**	**C . G**	**G . C**
Thymidine	37	10	24	26
I (Thymine)	20	10	14	15
II (2-Pyridone)	<5	n.d.	<5	n.d.
III (Acetyl)	n.o.	n.d.	n.o.	n.d.

Table 2 : 20 mM cacodylate, 100 mM NaCl, 10 mM $MgCl_2$, pH6.
n.d. and n.o. mean that the *Tm* was either not determined or observed, respectively.

Table 2 shows the *Tm* values of different Hoogsteen-motif triplexes (**10•9/8**). Here again the stability of the triplex was considerably decreased after the introduction of **I** at position Z of **8** (ΔTm = -17 °C). In contrast to the duplex case, the incorporation of a 2-pyridone (**II**) or an acetyl group (**III**) within the TFO resulted in a dramatically reduced stability, since the *Tm* of the triple helix formed with **8** (Z=**II**) was below 5 °C and could not be detected in the case of **8** (Z=**III**).

It is worthy of note that, to some extent, the thymine residue (Z=**I**) of the above backbone modified oligonucleotide still retains specific recognition of adenine when the latter is already engaged in a Watson-Crick T•A base pair. This can be deduced from the *Tm* values which were significantly decreased when thymine was facing other base pairs (A•T, G•C and C•G). Taken altogether these data indicate that with the above acyclic construction i) the thymine residue could specifically recognize adenine through Hoogsteen hydrogen bonds, ii) the flexible serinol backbone could account for the decreased thermal stability.

Acknowledgments: We thank Dr. M. Thomas for oligonucleotide syntheses and ANRS for financial support.

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(11) All new compounds showed satisfactory analytical and spectral data. Selected data are as follow: **5a**: mp (ether): 115-117°C; [α]$_D$ +12.3° (*c* 1.01 in CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.77 (s, 3H, Me), 3.21 (br d, 2H, *J* = 4.71 Hz, CH_2OH), 3.70 (m, 2H, CH_2ODMT), 3.76 (s, 6H, OMe), 4.12 (m, 1H, CHN), 4.15 and 4.36 (dd, 2H, *J* = 15.7 Hz, CH_2CO), 6.80 and 7.28 (dd, 8H, *J* = 8.77 Hz, DMT), 7.10-7.60 (m, 6H, H-6, DMT), 9.32 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 12.26, 50.28, 51.71, 55.26, 62.54, 62.76, 86.34, 110.95, 113.25, 126.93, 127.93, 128.12, 130.07, 135.81, 141.16, 144.74, 151.66, 158.57, 164.71, 167.04; MS (IC) 560 (MH^+); Anal. Calc. for $C_{31}H_{33}N_3O_7$: C 66.53%, H 5.94%, N 7.51%; found: C 66.31%, H 6.14%, N 7.35%. **5b**: mp (ether): 78-80°C; [α]$_D$ +8.6° (*c* 1.03 in CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.30 (d, 2H, *J* = 4.15 Hz, CH_2OH), 3.69 (m, 2H, CH_2ODMT), 3.78 (s, 6H, OMe), 4.20 (m, 1H, CHN), 4.54 (br s, 2H, CH_2CO), 6.23 (m, 1H, H-5), 6.56 (d, 1H, *J* = 9.82 Hz, H-3), 6.80 and 7.35 (dd, 8H, *J* = 8.69 Hz, DMT), 7.10-7.50 (m, 7H, H-4, H-6, DMT); ^{13}C NMR ($CDCl_3$) δ 51.78, 52.93, 55.18, 62.57, 62.70, 86.17, 106.73, 113.13, 120.53, 126.75, 127.72, 127.81, 128.05, 129.17, 129.99, 135.76, 138.58, 140.51, 144.70, 158.44, 162.71, 167.20; MS (IC) 529 (MH^+); Anal. Calc. for $C_{31}H_{32}N_2O_6$: C 70.44%, H 6.10%, N 5.30%; found: C 70.63%, H 6.42%, N 5.56%. **5c**: foam ; [α]$_D$ +1.1° (*c* 1.06 in CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.96 (s, 3H, Me), 2.75 (br s, 1H, OH), 3.33(t, 2H, *J* = 4.32, CH_2OH), 3.70 (m, 2H, CH_2ODMT), 3.75 (s, 6H, OMe), 4.07 (m, 1H, CHN), 5.96 (d, 1H, *J* = 4.86 Hz, NHAc), 6.83 and 7.31 (dd, 8H, *J* = 8.75 Hz, DMT), 7.15-7.50 (m, 5H, DMT); ^{13}C NMR ($CDCl_3$) δ 23.20, 51.53, 55.18, 62.85, 63.27, 86.39, 113.22, 126.91, 127.90, 127.96, 129.93, 135.66, 144.57, 158.54, 170.63; MS (IC) 436 (MH^+); Anal. Calc. for $C_{26}H_{29}NO_5$: C 71.70%, H 6.71%, N 3.22%; found: C 71.52%, H 6.85%, N 2.12%.